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(FILE 'HOME' ENTERED AT 10:26:43 ON 24 JUL 2006)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, JAPIO' ENTERED AT 10:27:00 ON 24 JUL 2006

L1 11983 S (NEURAL CELL ADHESION MOLECULE)
L2 41 S L1 AND STROKE?
L3 70234 S (VASCULAR ENDOTHELIAL GROWTH FACTOR)
L4 83 S L1 AND L3
L5 6 S L4 AND STROKE?
L6 6 DUPLICATE REMOVE L5 (0 DUPLICATES REMOVED)
L7 3833 S (B TYPE NATRIURETIC PEPTIDE)
L8 185 S L7 AND STROKE?
L9 5 S L7 AND VASOSPASM?
L10 5 S L9 AND L8
L11 5 DUPLICATE REMOVE L10 (0 DUPLICATES REMOVED)
L12 4 S L2 AND VASOSPASM?
L13 4 DUPLICATE REMOVE L12 (0 DUPLICATES REMOVED)
L14 14 S L2 AND PD<2003
L15 11 DUPLICATE REMOVE L14 (3 DUPLICATES REMOVED)
L16 11 S L15 NOT L13
L17 627 S L3 AND STROKE?
L18 7 S L17 AND VASOSPASM?
L19 3 S L18 NOT L13
L20 69 S L17 AND REVIEW?
L21 47 DUPLICATE REMOVE L20 (22 DUPLICATES REMOVED)
L22 19 S L21 AND PD<2003
L23 19 S L22 NOT L19
L24 19 S L23 NOT L13
L25 133 DUPLICATE REMOVE L8 (52 DUPLICATES REMOVED)
L26 18 S L25 AND PD<2003
L27 18 S L26 NOT L13
L28 18 S L27 NOT L19

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L26 18 S L25 AND PD<2003
L27 18 S L26 NOT L13
L28 18 S L27 NOT L19

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L28 ANSWER 7 OF 18 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
 AN 2000:73430 BIOSIS
 DN PREV200000073430
 TI Sustained hemodynamic effects of an infusion of nesiritide (human
 b-type natriuretic peptide) in heart
 failure: A randomized, double-blind, placebo-controlled clinical trial.
 AU Mills, Roger M. [Reprint author]; LeJemtel, Thierry H.; Horton, Darlene
 P.; Liang, Chang-seng; Lang, Roberto; Silver, Marc A.; Lui, Charles;
 Chatterjee, Kanu
 CS Desk F-15, Department of Cardiology, Cleveland Clinic Foundation, 9500
 Euclid Avenue, Cleveland, OH, USA
 SO Journal of the American College of Cardiology, (July, 1999) Vol.
 34, No. 1, pp. 155-162. print.
 CODEN: JACCDI. ISSN: 0735-1097.
 DT Article
 LA English
 ED Entered STN: 16 Feb 2000
 Last Updated on STN: 3 Jan 2002
 AB OBJECTIVES The goal of this study was to further define the role of
 nesiritide (human b-type natriuretic
 peptide) in the therapy of decompensated heart failure (HF) by
 assessing the hemodynamic effects of three doses (0.015, 0.03 and 0.06
 mug/kg/min) administered by continuous intravenous (IV) infusion over 24 h
 as compared with placebo. BACKGROUND Previous studies have shown
 beneficial hemodynamic, neurohormonal and renal effects of bolus dose and
 6-h infusion administration of nesiritide in HF patients. Longer term
 safety and efficacy have not been studied. METHODS This randomized,
 double-blind, placebo-controlled multicenter trial enrolled subjects with
 symptomatic HF and systolic dysfunction (left ventricular ejection
 fraction ltoreq35%). Central hemodynamics were assessed at baseline,
 during a 24-h IV infusion and for 4 h postinfusion. RESULTS One hundred
 three subjects with New York Heart Association class II (6%), III (61%) or
 IV (33%) HF were enrolled. Nesiritide produced significant reductions in
 pulmonary wedge pressure (27% to 39% decrease by 6 h), mean right atrial
 pressure and systemic vascular resistance, along with significant
 increases in cardiac index and stroke volume index, with no
 significant effect on heart rate. Beneficial effects were evident at 1 h
 and were sustained throughout the 24-h infusion. CONCLUSIONS The rapid
 and sustained beneficial hemodynamic effects of nesiritide observed in
 this study support its use as a first-line IV therapy for patients with
 symptomatic decompensated HF.
 CC Pharmacology - General 22002
 Pathology - Therapy 12512
 Cardiovascular system - General and methods 14501
 IT Major Concepts
 Cardiovascular Medicine (Human Medicine, Medical Sciences);
 Pharmacology
 IT Diseases
 heart failure: heart disease, treatment
 Heart Failure, Congestive (MeSH)
 IT Chemicals & Biochemicals
 nesiritide [human b-type natriuretic
 peptide]: cardiovascular-drug, continuous intravenous infusion,
 hemodynamic effects
 IT Miscellaneous Descriptors
 cardiac index; mean right atrial pressure; pulmonary wedge pressure;
 stroke volume; systemic vascular resistance; systolic
 dysfunction
 ORGN Classifier
 Hominidae 86215
 Super Taxa
 Primates; Mammalia; Vertebrata; Chordata; Animalia
 Organism Name

L28 ANSWER 7 OF 18 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
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 Cardiovascular system - General and methods 14501
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 IT Diseases
 heart failure: heart disease, treatment
 Heart Failure, Congestive (MeSH)
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 dysfunction
 ORGN Classifier
 Hominidae 86215
 Super Taxa
 Primates; Mammalia; Vertebrata; Chordata; Animalia
 Organism Name

human: patient

Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 124584-08-3 (nesiritide)

124584-08-3 (human b-type natriuretic
peptide)

human: patient

Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 124584-08-3 (nesiritide)
124584-08-3 (human b-type natriuretic
peptide)

ANSWER 8 OF 18 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
AN 1993:475317 BIOSIS
DN PREV199396108917
TI Chronic ethanol treatment increases the circulating plasma levels of
B-type natriuretic peptide (BNP-45)
in the rat.
AU Wigle, D. A.; Pang, S. C. [Reprint author]; Watson, J. D.; Sarda, I. R.;
Radakovic, N. N.; Flynn, T. G.
CS Dep. Anat., Queen's Univ., Kingston K7L 3N6, Canada
SO American Journal of Hypertension, (1993) Vol. 6, No. 8, pp.
719-722.
CODEN: AJHYE6. ISSN: 0895-7061.
DT Article
LA English
ED Entered STN: 22 Oct 1993
Last Updated on STN: 23 Oct 1993
AB Chronic ethanol ingestion is associated with a number of cardiovascular
disorders, including stroke, heart failure, and hypertension.
Given that the regulation of A-type natriuretic peptide (ANP) and
B-type natriuretic peptide (BNP) is
known to be altered in both congestive heart failure and essential
hypertension, we have investigated the regulation of BNP under the
influence of ethanol ingestion. Sprague-Dawley rats were given ethanol in
drinking fluid for a 6-week period, while a weight-matched
liquid-restricted group received an equivalent volume of ethanol-free
solution. Plasma BNP levels were increased in ethanol-treated animals
relative to both liquid-restricted and normal control groups. No changes
in cardiac BNP gene expression were observed, but an increased trend in
atrial tissue BNP levels was evident. No changes in either the mRNA,
tissue, or plasma levels of ANP were evident. These results suggest a
differential regulation of natriuretic peptides under the influence of
ethanol, and implicate chronic ethanol ingestion as a further clinical
condition under which the plasma levels of a natriuretic peptide may be
elevated.
CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Metabolism - Proteins, peptides and amino acids 13012
Nutrition - Pathogenic diets 13216
Cardiovascular system - Physiology and biochemistry 14504
Blood - Blood and lymph studies 15002
Endocrine - Neuroendocrinology 17020
Nervous system - Physiology and biochemistry 20504
IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Cardiovascular System
(Transport and Circulation); Endocrine System (Chemical Coordination
and Homeostasis); Metabolism; Nervous System (Neural Coordination);
Nutrition
IT Chemicals & Biochemicals
ETHANOL
IT Miscellaneous Descriptors
CARDIAC MYOCYTE; CYCLIC AMP; HEART RATE; NORADRENALINE; NOREPINEPHRINE;
REGULATORY PEPTIDE
ORGN Classifier
Canidae 85765
Super Taxa
Carnivora; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
dog
Taxa Notes
Animals, Carnivores, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman
Mammals, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa

ANSWER 8 OF 18 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
AN 1993:475317 BIOSIS
DN PREV199396108917
TI Chronic ethanol treatment increases the circulating plasma levels of
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in the rat.
AU Wigle, D. A.; Pang, S. C. [Reprint author]; Watson, J. D.; Sarda, I. R.;
Radakovic, N. N.; Flynn, T. G.
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SO American Journal of Hypertension, (1993) Vol. 6, No. 8, pp.
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Blood - Blood and lymph studies 15002
Endocrine - Neuroendocrinology 17020
Nervous system - Physiology and biochemistry 20504
IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Cardiovascular System
(Transport and Circulation); Endocrine System (Chemical Coordination
and Homeostasis); Metabolism; Nervous System (Neural Coordination);
Nutrition
IT Chemicals & Biochemicals
ETHANOL
IT Miscellaneous Descriptors
CARDIAC MYOCYTE; CYCLIC AMP; HEART RATE; NORADRENALINE; NOREPINEPHRINE;
REGULATORY PEPTIDE
ORGN Classifier
Canidae 85765
Super Taxa
Carnivora; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
dog
Taxa Notes
Animals, Carnivores, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman
Mammals, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa

Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name

rat

Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,
Rodents, Vertebrates

RN 64-17-5 (ETHANOL)

Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
rat

Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,
Rodents, Vertebrates

RN 64-17-5 (ETHANOL)

ANSWER 13 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

AN 1998:552411 CAPLUS

DN 129:300659

ED Entered STN: 01 Sep 1998

TI Angiogenesis and stroke

AU Greenberg, David A.

CS Depts. of Neurology and Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213, USA

SO Drug News & Perspectives (1998), 11(5), 265-270

CODEN: DNPEED; ISSN: 0214-0934

PB Prous Science

DT Journal; General Review

LA English

CC 14-0 (Mammalian Pathological Biochemistry)

AB Stroke results from focal cerebral ischemia due to occlusion of a cerebral blood vessel, usually an artery. Where ischemia is chronic or intermittent, collateral circulation may develop by enlargement of preexisting anastomotic channels or sprouting of new capillaries from existing vessels (angiogenesis). Angiogenesis has 3 attributes of particular interest in relation to cerebral vascular disease: (1) it is the principal mechanism by which the brain is vascularized; (2) unlike vasculogenesis, it continues in adulthood; and (3) as in other tissues, it can be induced in the CNS by hypoxia or ischemia. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis. The angiopoietins, Ang-1 and Ang-2, and their common receptor, Tie-2 or Tek, constitute another signaling system that regulates angiogenesis, and which interacts with VEGF. Four recent studies provide evidence for the induction of angiogenesis, VEGF and VEGF receptor expression in exptl. models of cerebral ischemia. Further understanding of the role of VEGF, VEGF receptors and angiogenesis in the brain's response to ischemia may have implications for prognosis and treatment in stroke. All this and more was reviewed with 87 refs.

ST review angiogenesis stroke

IT Angiogenesis

(and stroke)

IT Brain, disease

(stroke; angiogenesis and)

RE.CNT 87 THERE ARE 87 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Asahara, T; Science 1997, V275, P964 CAPLUS

(2) Banai, S; Cardiovasc Res 1994, V28, P1176 CAPLUS

(3) Banai, S; Circulation 1994, V89, P2183 CAPLUS

(4) Barnett, H; N Engl J Med 1995, V332, P238 MEDLINE

(5) Battegay, E; J Mol Med 1995, V73, P333 CAPLUS

(6) Bauters, C; Am J Physiol 1994, V267, PH1263 CAPLUS

(7) Beck, L; FASEB J 1997, V11, P365 CAPLUS

(8) Breier, G; Thromb Haemost 1997, V78, P678 CAPLUS

(9) Brogi, E; J Clin Invest 1996, V97, P469 CAPLUS

(10) Bronner, L; N Engl J Med 1995, V333, P1392 MEDLINE

(11) Bunn, H; Physiol Rev 1996, V76, P839 CAPLUS

(12) Carmeliet, P; Nature 1996, V380, P435 CAPLUS

(13) Chen, H; Stroke 1994, V25, P1651 CAPLUS

(14) Cobbs, C; Neurosci Lett, in press 1998

(15) Criscuolo, G; J Neurosurg 1989, V71, P884 CAPLUS

(16) Davis, S; Cell 1996, V87, P1161 CAPLUS

(17) de Vries, C; Science 1992, V255, P989 CAPLUS

(18) Ferrara, N; Nature 1996, V380, P439 CAPLUS

(19) Folkman, J; N Engl J Med 1995, V333, P1757 MEDLINE

(20) Folkman, J; Nat Med 1995, V1, P27 CAPLUS

(21) Folkman, J; Nature 1980, V288, P551 MEDLINE

(22) Fong, G; Nature 1995, V376, P66 CAPLUS

(23) Fox, J; Circulation 1996, V94, P3065 MEDLINE

(24) Goldberg, M; J Biol Chem 1994, V269, P4355 CAPLUS

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AN 1998:552411 CAPLUS

DN 129:300659

ED Entered STN: 01 Sep 1998

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AU Greenberg, David A.

CS Depts. of Neurology and Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213, USA

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ST review angiogenesis stroke

IT Angiogenesis
(and stroke)

IT Brain, disease
(stroke; angiogenesis and)

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- (38) Jeffs, B; Nat Genet 1997, V16, P364 CAPLUS
- (39) Jeltsch, M; Science 1997, V276, P1423 CAPLUS
- (40) Joukov, V; EMBO J 1996, V15, P290 CAPLUS
- (41) Keck, P; Science 1989, V246, P1309 CAPLUS
- (42) Keyt, B; J Biol Chem 1996, V271, P5638 CAPLUS
- (43) Kim, K; Nature 1993, V362, P841 CAPLUS
- (44) Knighton, D; Surgery 1981, V90, P262 CAPLUS
- (45) Koistinaho, J; NeuroReport 1997, V8, Pi CAP

- (25) Greenberg, D; FASEB J 1998, V12, PA668
- (26) Hanahan, D; Science 1997, V277, P48 CAPLUS
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- (39) Jeltsch, M; Science 1997, V276, P1423 CAPLUS
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- (44) Knighton, D; Surgery 1981, V90, P262 CAPLUS
- (45) Koistinaho, J; NeuroReport 1997, V8, Pi CAP

ANSWER 14 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

AN 1998:374091 CAPLUS
DN 129:159397
ED Entered STN: 19 Jun 1998
TI Genetic analysis of the coagulation and plasminogen systems: implications
in blood vessel formation, hemostasis, thrombosis, stroke,
restenosis, aneurysm formation and atherosclerosis
AU Carmeliet, Peter
CS Center for Transgene Technology and Gene Therapy, Flanders Interuniversity
Institute for Biotechnology, KU Leuven, Louvain, B-3000, Belg.
SO International Congress Series (1997), 1129(Recent Progress in
Blood Coagulation and Fibrinolysis), 95-105
CODEN: EXMDA4; ISSN: 0531-5131
PB Elsevier Science B.V.
DT Journal; General Review
LA English
CC 13-0 (Mammalian Biochemistry)
Section cross-reference(s): 2, 14
AB A review, with 88 refs., aims at integrating the pleiotropic
roles of the VEGF, coagulation and plasminogen systems in vascular biol.,
as deduced from targeted gene manipulation (gene inactivation or gene
transfer) studies in the mouse.
ST review VEGF coagulation plasminogen thrombosis atherosclerosis
IT Aneurysm
Angiogenesis
Atherosclerosis
Blood coagulation
(genetic anal. of coagulation and plasminogen systems with implications
in blood vessel formation, hemostasis, thrombosis, stroke,
restenosis, aneurysm formation and atherosclerosis)
IT Artery, disease
(restenosis; genetic anal. of coagulation and plasminogen systems with
implications in blood vessel formation, hemostasis, thrombosis,
stroke, restenosis, aneurysm formation and atherosclerosis)
IT 9001-91-6, Plasminogen 127464-60-2, Vascular
endothelial growth factor
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(genetic anal. of coagulation and plasminogen systems with implications
in blood vessel formation, hemostasis, thrombosis, stroke,
restenosis, aneurysm formation and atherosclerosis)

RE.CNT 88 THERE ARE 88 CITED REFERENCES AVAILABLE FOR THIS RECORD

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- (7) Bolton-Maggs, P; Blood Rev 1995, V9, P65 MEDLINE
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- (11) Bugge, T; Proc Natl Acad Sci USA 1996, V93, P6258 CAPLUS
- (12) Capecchi, M; Sci Am 1994, V270, P52 MEDLINE
- (13) Carmeliet, P; Am J Pathol 1997, V150, P761 MEDLINE
- (14) Carmeliet, P; Ann NY Acad Sci 1995, V748P, P367
- (15) Carmeliet, P; Ann NY Acad Sci in press
- (16) Carmeliet, P; Fibrinolysis, abstract 57 1996, V10(suppl 3)
- (17) Carmeliet, P; J Clin Invest 1993, V92, P2746 CAPLUS
- (18) Carmeliet, P; J Clin Invest 1993, V92, P2756 CAPLUS
- (19) Carmeliet, P; J Clin Invest 1997, V99, P2746
- (20) Carmeliet, P; Nature 1994, V368, P419 CAPLUS
- (21) Carmeliet, P; Nature 1996, V383, P73 CAPLUS
- (22) Carmeliet, P; Nature (London) 1996, V380(6573), P435 CAPLUS

ANSWER 14 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

AN 1998:374091 CAPLUS

DN 129:159397

ED Entered STN: 19 Jun 1998

TI Genetic analysis of the coagulation and plasminogen systems: implications in blood vessel formation, hemostasis, thrombosis, stroke, restenosis, aneurysm formation and atherosclerosis

AU Carmeliet, Peter

CS Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, KU Leuven, Louvain, B-3000, Belg.

SO International Congress Series (1997), 1129(Recent Progress in Blood Coagulation and Fibrinolysis), 95-105
CODEN: EXMDA4; ISSN: 0531-5131

PB Elsevier Science B.V.

DT Journal; General Review

LA English

CC 13-0 (Mammalian Biochemistry)
Section cross-reference(s): 2, 14

AB A review, with 88 refs., aims at integrating the pleiotropic roles of the VEGF, coagulation and plasminogen systems in vascular biol., as deduced from targeted gene manipulation (gene inactivation or gene transfer) studies in the mouse.

ST review VEGF coagulation plasminogen thrombosis atherosclerosis

IT Aneurysm

Angiogenesis

Atherosclerosis

Blood coagulation

(genetic anal. of coagulation and plasminogen systems with implications in blood vessel formation, hemostasis, thrombosis, stroke, restenosis, aneurysm formation and atherosclerosis)

IT Artery, disease

(restenosis; genetic anal. of coagulation and plasminogen systems with implications in blood vessel formation, hemostasis, thrombosis, stroke, restenosis, aneurysm formation and atherosclerosis)

IT 9001-91-6, Plasminogen 127464-60-2, Vascular endothelial growth factor

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(genetic anal. of coagulation and plasminogen systems with implications in blood vessel formation, hemostasis, thrombosis, stroke, restenosis, aneurysm formation and atherosclerosis)

RE.CNT 88 THERE ARE 88 CITED REFERENCES AVAILABLE FOR THIS RECORD

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(3) Aoki, N; Blood Rev 1989, V3, P11 MEDLINE

(4) Bi, L; Nature Genetics 1995, V10, P119 CAPLUS

(5) Bick, R; Sem Thromb Hemostas 1994, V20, P109 MEDLINE

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(7) Bolton-Maggs, P; Blood Rev 1995, V9, P65 MEDLINE

(8) Broze, G; Annu Rev Med 1995, V46, P103 CAPLUS

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(11) Bugge, T; Proc Natl Acad Sci USA 1996, V93, P6258 CAPLUS

(12) Capecchi, M; Sci Am 1994, V270, P52 MEDLINE

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(15) Carmeliet, P; Ann NY Acad Sci in press

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(17) Carmeliet, P; J Clin Invest 1993, V92, P2746 CAPLUS

(18) Carmeliet, P; J Clin Invest 1993, V92, P2756 CAPLUS

(19) Carmeliet, P; J Clin Invest 1997, V99, P2746

(20) Carmeliet, P; Nature 1994, V368, P419 CAPLUS

(21) Carmeliet, P; Nature 1996, V383, P73 CAPLUS

(22) Carmeliet, P; Nature (London) 1996, V380(6573), P435 CAPLUS

- (23) Carmeliet, P; Submitted
- (24) Chapman, H; Biochem J 1984, V222, P721 CAPLUS
- (25) Chapman, H; J Clin Invest 1984, V73, P806 CAPLUS
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- (29) Conolly, A; Nature 1996, V381, P516
- (30) Contrino, J; Nat Med 1996, V2, P209 CAPLUS
- (31) Coughlin, S; Semin Hematol 1994, V31, P270 CAPLUS
- (32) Cui, J; Nature 1996, V384 CAPLUS

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- (31) Coughlin, S; Semin Hematol 1994, V31, P270 CAPLUS
- (32) Cui, J; Nature 1996, V384 CAPLUS

ANSWER 7 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

AN 2001:154876 CAPLUS

DN 135:178958

ED Entered STN: 05 Mar 2001

TI The modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia

AU Fox, Gerard B.; Kjoller, Connie; Murphy, Keith J.; Regan, Ciaran M.

CS Department of Pharmacology, Conway Institute, National University of Ireland, Dublin, 4, Ire.

SO Journal of Neuropathology and Experimental Neurology (2001), 60(2), 132-140

CODEN: JNENAD; ISSN: 0022-3069

PB American Association of Neuropathologists, Inc.

DT Journal

LA English

CC 14-10 (Mammalian Pathological Biochemistry)

AB To investigate the role of polysialylated neural cell adhesion mol. (NCAM PSA)-mediated plasticity after injury, the authors examined the temporal and spatial expression of NCAM PSA immunoreactivity in the medial temporal lobe following global ischemia. Male Mongolian gerbils were subjected to bilateral common carotid artery occlusion for 5 min and killed at increasing times post-occlusion. The well-characterized delayed CA1 pyramidal cell death was observed 5-7 days post-occlusion. At post-occlusion days 1-2 there was a small but significant increase of NCAM PSA-pos. hippocampal granule cells followed by an equally significant decrease at post-occlusion day 5. In contrast, a substantial increase in glial PSA expression was observed in all hippocampal regions at 1-7 days post-occlusion that was associated generally with stellate astroglia and specifically with the radial processes of glia traversing the granule cell layer of the dentate gyrus. Administration of the glutamate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline significantly blocked the ischemia-induced modulation of neuronal and glial NCAM PSA expression. Astroglial NCAM polysialylation became attenuated by 35 days post-occlusion except in the CA1 area of cell death. The temporal and regional pattern of polysialylated NCAM expression in the ischemic gerbil hippocampus implicates this neuroplastic marker in mechanisms of neurotrophic-dependent repair/remodeling that ensue following transient interruption of blood flow.

ST NCAM polysialylation transient global ischemia neuron glia

IT Cell adhesion molecules

RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)

(N-CAM; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Artery, disease

(carotid, occlusion; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain

(dentate gyrus; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Nerve

(granule cell, hippocampal; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain

(hippocampus, granular cell layer; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain

(hippocampus, sector CA1, pyramidal cell layer; modulations of NCAM polysialylation state that follow transient global ischemia are brief

ANSWER 7 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

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SO Journal of Neuropathology and Experimental Neurology (2001), 60(2), 132-140

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ST NCAM polysialylation transient global ischemia neuron glia

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IT Brain

(hippocampus, granular cell layer; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain

(hippocampus, sector CA1, pyramidal cell layer; modulations of NCAM polysialylation state that follow transient global ischemia are brief

on neurons but enduring on glia)

IT Brain
(hippocampus; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain, disease
(ischemia, transient; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Astrocyte
Cell death
Neuroglia
(modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Synaptic plasticity
(modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia in relation to)

IT Nerve
(neuron; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Sialylation
(polysialylation; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Nerve
(pyramidal cell, hippocampal; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain, disease
(stroke; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia in relation to)

IT 56-86-0, L-Glutamic acid, biological studies
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia in relation to)

RE.CNT 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD

on neurons but enduring on glia)

IT Brain
(hippocampus; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

IT Brain, disease
(ischemia, transient; modulations of NCAM polysialylation state that follow transient global ischemia are brief on neurons but enduring on glia)

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RE.CNT 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L/COOK 7/24/06
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FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, JAPIO' ENTERED AT 08:40:03 ON 24
JUL 2006

L1 164 S VASOPASM?
L2 4 S (SUBARRACHNOID HEMORRHAGE)
L3 0 S L2 AND L1
L4 152 DUPLICATE REMOVE L1 (12 DUPLICATES REMOVED)
L5 142 S L4 AND PD<2003
L6 2 S L5 AND STROKE?
L7 11 S L4 AND REVIEW?
L8 11 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)

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L5 142 S L4 AND PD<2003
L6 2 S L5 AND STROKE?
L7 11 S L4 AND REVIEW?
L8 11 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)

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Volume 35, Issue 1, January 1991, Pages 20-29

doi:10.1016/0090-3019(91)90197-H [Cite or Link Using DOI](#)
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Cerebrospinal fluid endothelin-1 and endothelin-3 levels in normal and neurosurgical patients: A clinical study and literature review

Gary E. Kraus M.D. , Richard D. Bucholz M.D., Kong-Woo Yoon M.D., Mark M. Knuepfer Ph.D. and Kenneth R. Smith, Jr. M.D.


Division of Neurosurgery, Departments of Surgery and Pharmacology, St. Louis University Medical Center, St. Louis, Missouri, USA

Received 3 July 1990; accepted 1 August 1990. Available online 17 March 2004.

Abstract

Endothelins are a family of structurally related, potent, long-lasting vasoconstrictor peptides. There are no established normal human levels of endothelin-1 or endothelin-3 in the cerebrospinal fluid. We measured cerebrospinal fluid endothelin-1 and endothelin-3 levels in five groups of patients: normal controls, patients with subarachnoid hemorrhage and cerebral vasospasm, patients with severe head injuries, patients undergoing temporal lobectomy for intractable epilepsy, and a patient with a gunshot injury to the thoracic spine. Endothelin-3 levels were significantly elevated in patients with subarachnoid hemorrhage and may participate in cerebral vasospasm and subsequent neurologic deterioration.

Author Keywords: Author Keywords: Endothelin-1; Endothelin-3; Cerebral vasospasm; Subarachnoid hemorrhage; Cerebrospinal fluid; Aneurysm

 Corresponding author. Address reprint requests to: Gary E. Kraus, M.D., Division of Neurosurgery, St. Louis University Medical Center, 3635 Vista Avenue at Grand Boulevard, P.O. Box 15250, St. Louis, Missouri 63110-0250.

Surgical Neurology

Volume 35, Issue 1 , January 1991, Pages 20-29

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AN 1991:116713 BIOSIS

DN PREV199191064103; BA91:64103

TI CEREBROSPINAL FLUID ENDOTHELIN-1 AND ENDOTHELIN-3 LEVELS IN NORMAL AND NEUROSURGICAL PATIENTS A CLINICAL STUDY AND LITERATURE REVIEW.

AU KRAUS G E [Reprint author]; BUCHOLZ R D; YOON K-W; KNEUPFER M M; SMITH K R JR

CS DIV NEUROSURGERY, ST LOUIS UNIV MED CENT, 3635 VISTA AVE AT GRAND BLVD, PO BOX 15250, ST LOUIS, MO 63110-0250, USA

SO Surgical Neurology, (1991) Vol. 35, No. 1, pp. 20-29.
ISSN: 0090-3019.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 27 Feb 1991
Last Updated on STN: 27 Feb 1991

AB Endothelins are a family of structurally related, potent, long-lasting vasoconstrictor peptides. There are no established normal human levels of endothelin-1 or endothelin-3 in the cerebrospinal fluid. We measured cerebrospinal fluid endothelin-1 and endothelin-3 levels in five groups of patients: normal controls, patients with subarachnoid hemorrhage and cerebral vasospasm, patients with severe head injuries, patients undergoing temporal lobectomy for intractable epilepsy, and a patient with a gunshot injury to the thoracic spine. Endothelin-3 levels were significantly elevated in patients with subarachnoid hemorrhage and may participate in cerebral vasospasm and subsequent neurologic deterioration.

CC Biochemistry studies - Proteins, peptides and amino acids 10064
External effects - Physical and mechanical effect 10612
Anatomy and Histology - Surgery 11105
Chordate body regions - Head 11304
Chordate body regions - Back and buttocks 11310
Metabolism - Proteins, peptides and amino acids 13012
Cardiovascular system - Blood vessel pathology 14508
Blood - Other body fluids 15010
Endocrine - Neuroendocrinology 17020
Nervous system - General and methods 20501
Nervous system - Physiology and biochemistry 20504
Nervous system - Pathology 20506

IT Major Concepts
Cardiovascular Medicine (Human Medicine, Medical Sciences); Endocrine System (Chemical Coordination and Homeostasis); Metabolism; Nervous System (Neural Coordination); Neurology (Human Medicine, Medical Sciences); Physiology; Surgery (Medical Sciences)

IT Miscellaneous Descriptors
HUMAN VASOCONSTRICTOR PEPTIDE SUBARACHNOID HEMORRHAGE

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AN 1991:116713 BIOSIS

DN PREV199191064103; BA91:64103

TI CEREBROSPINAL FLUID ENDOTHELIN-1 AND ENDOTHELIN-3 LEVELS IN NORMAL AND NEUROSURGICAL PATIENTS A CLINICAL STUDY AND LITERATURE REVIEW.

AU KRAUS G E [Reprint author]; BUCHOLZ R D; YOON K-W; KNEUPFER M M; SMITH K R JR

CS DIV NEUROSURGERY, ST LOUIS UNIV MED CENT, 3635 VISTA AVE AT GRAND BLVD, PO BOX 15250, ST LOUIS, MO 63110-0250, USA

SO Surgical Neurology, (1991) Vol. 35, No. 1, pp. 20-29.
ISSN: 0090-3019.

DT Article

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LA ENGLISH

ED Entered STN: 27 Feb 1991
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AB Endothelins are a family of structurally related, potent, long-lasting vasoconstrictor peptides. There are no established normal human levels of endothelin-1 or endothelin-3 in the cerebrospinal fluid. We measured cerebrospinal fluid endothelin-1 and endothelin-3 levels in five groups of patients: normal controls, patients with subarachnoid hemorrhage and cerebral vasospasm, patients with severe head injuries, patients undergoing temporal lobectomy for intractable epilepsy, and a patient with a gunshot injury to the thoracic spine. Endothelin-3 levels were significantly elevated in patients with subarachnoid hemorrhage and may participate in cerebral vasospasm and subsequent neurologic deterioration.

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Nervous system - General and methods 20501
Nervous system - Physiology and biochemistry 20504
Nervous system - Pathology 20506

IT Major Concepts
Cardiovascular Medicine (Human Medicine, Medical Sciences); Endocrine System (Chemical Coordination and Homeostasis); Metabolism; Nervous System (Neural Coordination); Neurology (Human Medicine, Medical Sciences); Physiology; Surgery (Medical Sciences)

IT Miscellaneous Descriptors
HUMAN VASOCONSTRICTOR PEPTIDE SUBARACHNOID HEMORRHAGE

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L/cook 7/24/06.

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(FILE 'HOME' ENTERED AT 10:26:43 ON 24 JUL 2006)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, JAPIO' ENTERED AT 10:27:00 ON 24 JUL 2006

L1 11983 S (NEURAL CELL ADHESION MOLECULE)
L2 41 S L1 AND STROKE?
L3 70234 S (VASCULAR ENDOTHELIAL GROWTH FACTOR)
L4 83 S L1 AND L3
L5 6 S L4 AND STROKE?
L6 6 DUPLICATE REMOVE L5 (0 DUPLICATES REMOVED)
L7 3833 S (B TYPE NATRIURETIC PEPTIDE)
L8 185 S L7 AND STROKE?
L9 5 S L7 AND VASOSPASM?
L10 5 S L9 AND L8
L11 5 DUPLICATE REMOVE L10 (0 DUPLICATES REMOVED)

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ANSWER 8 OF 8 MEDLINE on STN
AN 2001380602 MEDLINE
DN PubMed ID: 11436351
TI Gene therapy with adenovirus-mediated glial cell line-derived neurotrophic factor and neural stem cells activation after ischemic brain injury.
AU Iwai M; Abe K; Kitagawa H; Hayashi T
CS Department of Neurology, Okayama University Medical School.
SO Human cell : official journal of Human Cell Research Society, (2001 Mar) Vol. 14, No. 1, pp. 27-38.
Journal code: 8912329. ISSN: 0914-7470.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200108
ED Entered STN: 3 Sep 2001
Last Updated on STN: 3 Sep 2001
Entered Medline: 30 Aug 2001
AB Recent advancements in molecular biology are made to expect the appearance of the new treatment of stroke patients. One is the administration of neurotrophic factors, and another is the use of neural stem cell. In this report, we performed two experiments. First experiment is administration of glial cell line-derived neurotrophic factor (GDNF) using an adenovirus vector into ischemic rat brain. A replication-defective adenoviral vector containing GDNF gene (Ad-GDNF) was directly injected into the cerebral cortex at 1 day before 90 min of transient middle cerebral artery occlusion (MCAO) in rats. Infarct volume of the Ad-GDNF injected group at 24 h after the transient MCAO was significantly smaller than that of vehicle or Ad-LacZ treated group. These results suggest that the successful exogenous GDNF gene transfer ameliorates the ischemic brain injury after transient MCAO in association with the reduction of apoptotic signals. Second one is the neural stem cell activation after transient ischemia. We investigated a possible expression of highly polysialylated neural cell adhesion molecule (PSA-NCAM) in gerbil hippocampus after 5 min of transient global ischemia in association to the proliferation of neural stem cell labeled with bromodeoxyuridine (BrdU). The number of PSA-NCAM positive cells increased in dentate gyrus (DG) at 10 and 20 days, and that of BrdU-labeled cells increased in DG at 5 and 10 days after the reperfusion. Immunofluorescence for PSA-NCAM and BrdU showed that a few cells per section were double labeled in DG only at 10 days after the reperfusion. These results suggest different chronological change of PSA-NCAM positive and BrdU-labeled cells in DG after transient ischemia.
CT Check Tags: Male
*Adenoviridae

ANSWER 1 OF 8 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

AN 2001:87059 BIOSIS

DN PREV200100087059

TI Increased forebrain subventricular zone neurogenesis and altered neuronal precursor migration in the adult rat brain after focal ischemic injury.

AU Parent, J. M. [Reprint author]; Vexler, Z. S.; Derugin, N.; Valentin, V. V.; Ferriero, D. M.

CS Univ. California, San Francisco, San Francisco, CA, USA

SO Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-114.9. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience. ISSN: 0190-5295.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LA English

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AB Neurogenesis persists in the adult rodent forebrain subventricular zone (SVZ). SVZ precursors migrate in a spatially restricted rostral migratory stream (RMS) to reach the olfactory bulb and differentiate into neurons. This study examined whether focal ischemia alters SVZ neurogenesis or precursor migration in the RMS. Focal cerebral infarcts were produced in adult rats by 90-120 min of right middle cerebral artery suture occlusion. S-phase cells were labeled with bromodeoxyuridine (BrdU) given twice daily 7-9 days after ischemia. At 21 d, BrdU-labeled cells, neuronal precursors and glia were identified by immunostaining for BrdU, polysialylated neural cell adhesion molecule (PSA-NCAM), neuron-specific beta-tubulin (TuJ1 antibody), vimentin or GFAP. BrdU-immunoreactivity (IR) increased in the rostral SVZ and RMS ipsilateral to infarcted frontal cortex and/or striatum compared to the contralateral side. Many BrdU-labeled cells were also found at the infarct edge or outside the SVZ or RMS. PSA-NCAM- and TuJ1-IR also increased in the SVZ and RMS ipsilateral to the stroke, and these cells did not co-express glial markers. PSA-NCAM-IR cells frequently appeared in chains extending from the SVZ or RMS towards the infarcted tissue, typically in close proximity to glial processes. These findings indicate that cerebral ischemia increases rostral SVZ neurogenesis. Furthermore, the directed migration of endogenous precursors out of the SVZ or RMS toward infarcted cortex suggests that these cells respond to an injury-induced chemoattractant.

CC Nervous system - Pathology 20506
General biology - Symposia, transactions and proceedings 00520
Cardiovascular system - Blood vessel pathology 14508
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IT Major Concepts
Nervous System (Neural Coordination)

IT Parts, Structures, & Systems of Organisms
brain: nervous system

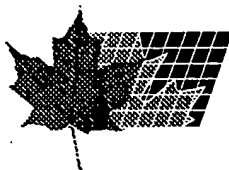
IT Diseases
focal cerebral infarction: nervous system disease

IT Diseases
focal ischemic injury: vascular disease

IT Miscellaneous Descriptors
forebrain subventricular zone neurogenesis; neuronal precursor migration; rostral migratory stream; Meeting Abstract

ORGN Classifier
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Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
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Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,

Rodents, Vertebrates

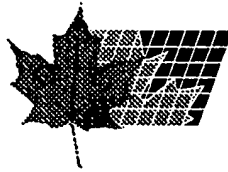


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(54) COMPOSES SE LIANT AUX N-CAM
(54) NCAM BINDING COMPOUNDS

(57) The invention provides novel compounds which are capable to stimulate the proliferation or/and the outgrowth from cells presenting the neural cell adhesion molecule (NCAM). Additionally, the invention relates to pharmaceutical compositions, medicaments and methods for treatment of normal, degenerated and damaged NCAM presenting cells.



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(54) **METHODE POUR DIAGNOSTIQUER ET DISTINGUER LES
ACCIDENTS VASCULAIRES CEREBRAUX ET DISPOSITIFS
DE DIAGNOSTIC UTILISES A CETTE FIN**

(54) **METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
AND DIAGNOSTIC DEVICES FOR USE THEREIN**

(57) A method for determining whether a subject has had a stroke and, if so, the type of stroke which includes analyzing the subject's body fluid for at least four selected markers of stroke, namely, myelin basic protein, S100 protein, neuronal specific enolase and a brain endothelial membrane protein such as thrombomodulin or a similar molecule. The data obtained from the analyses provide information as to the type of stroke, the onset of occurrence and the extent of brain damage and allow a physician to determine quickly the type of treatment required by the subject.

**METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
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ABSTRACT

5 A method for determining whether a subject has had a stroke and, if so, the type of stroke which includes analyzing the subject's body fluid for at least four selected markers of stroke, namely, myelin basic protein, S100 protein, neuronal specific enolase and a brain endothelial membrane protein such as thrombomodulin or a similar molecule. The data obtained from the analyses provide information as to the type of stroke, the onset of occurrence and the extent of brain damage and allow a physician to determine quickly the type of treatment required by the subject.

**METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
AND DIAGNOSTIC DEVICES FOR USE THEREIN**

BACKGROUND OF THE INVENTION

5 This application is directed to a method for diagnosing whether a subject has had a stroke and, if so, differentiating between the different types of stroke. More specifically, the method includes analyzing the subject's body fluid for at least four selected markers of stroke. There are also described diagnostic devices and kits for use in the method.

10 The impact of stroke on the health of human beings is very great when considered in terms of mortality and even more devastating when disability is considered. For example, stroke is the third leading cause of death in adults in the United States, after ischemic heart disease and all forms of cancer. For people who survive, stroke is the leading cause of disability. The direct medical costs due to
15 stroke and the cost of lost employment amount to billions of dollars annually. Approximately 85% of all strokes are ischemic (thrombotic and embolic) with the remainder being hemorrhagic.

 Stroke is an underserved market for both therapeutics and diagnostic techniques. In the United States alone over 700,000 people have strokes each year. A
20 multiple of that number would be suspected of having strokes with diagnostics only confirmed by expensive technology including computer-assisted tomography (CAT) scans and magnetic resonance imaging (MRI). However, these sophisticated technologies are not available in all hospitals and they are also not sensitive enough to diagnose ischemic stroke at an early stage.

25 Stroke is a clinical diagnosis made by a neurologist, usually as a consultation. Current methods for diagnosing stroke include symptom evaluation, medical history, chest X-ray, ECG (electrical heart activity), EEG (brain nerve cell activity), CAT scan to assess brain damage and MRI to obtain internal body visuals. A number of blood tests may be performed to search for internal bleeding. These include complete blood

count, prothrombin time, partial thromboplastin time, serum electrolytes and blood glucose.

Determining the immediate cause of a stroke can be difficult especially upon presentation where the diagnosis relies mainly on imaging techniques. Approximately 50% of cerebral infarctions are not visible on a CAT scan. Further, even though a CAT scan can be very sensitive for the identification of hemorrhagic stroke, it is not very sensitive for cerebral ischemia during evaluation of stroke and is usually positive at from 24 to 36 hours after onset of stroke. As a result a window of opportunity for rapid treatment would usually have expired once the current diagnostic techniques positively identify a stroke.

The treatment of stroke includes preventive therapies such as antihypertensive and antiplatelet drugs which control and reduce blood pressure and thus reduce the likelihood of stroke. Also, the development of thrombolytic drugs such as t-PA (tissue plasminogen activator) has provided a significant advance in the treatment of ischemic stroke victims but to be effective and minimize damage from acute stroke it is necessary to begin treatment very early, for example, within about three hours after the onset of symptoms. These drugs dissolve blood vessel clots which block blood flow to the brain and which are the cause of approximately 80% of strokes. However, these drugs can also present the side effect of increased risk of bleeding. Various neuroprotectors such as calcium channel antagonist can stop damage to the brain as a result of ischemic insult. The window of treatment for these drugs is typically broader than that for the clot dissolvers and they do not increase the risk of bleeding.

Diagnostic techniques for the early diagnosis of stroke and identification of the type of stroke are needed to allow the physician to prescribe the appropriate therapeutic drugs at an early stage in the cerebral event. Various markers for stroke are known and analytical techniques for the determination of such markers have been described in the art. As used herein the term "marker" refers to a protein or other molecule that is released from the brain during a cerebral ischemic or hemorrhagic event. Such markers include isoforms of proteins that are unique to the brain.

It has been reported in the literature that myelin basic protein (MBP) concentration, in cerebrospinal fluid (CSF) increases after sufficient damage to

neuronal tissue, head trauma and AIDS dementia. Further, it has been reported that ultrastructural immunocytochemistry studies using anti-MBP antibodies have shown that MBP is localized exclusively in the myelin sheath. Thus, it has been suggested the MBP levels in CSF or serum be used as a marker of cerebral damage in acute cerebrovascular disease. See Strand, T., et al., Brain and plasma proteins in spinal fluid as markers for brain damage and severity of stroke, *Stroke* (1984) 15; 138-144. The increase in MBP concentration in CSF is most evident in about four to five days after the onset of thrombotic stroke while in cerebral hemorrhage the increase was highest almost immediately after onset. See Garcia-Alex, A., et al., Neuron-specific enolase and myelin basic protein: Relationship of cerebrospinal fluid concentration to the neurologic condition of asphyxiated full-term infants, *Pediatrics* (1994) 93; 234-240. It has also been found that patients with transitory ischemic attack (TIA) had normal CSF values for MBP while those with cerebral infarction and hemorrhage had elevated values. In cerebral infarction there was a significant increase in MBP concentration in CSF from the first to second lumbar puncture while patients with intracerebral hemorrhage had reached already markedly elevated levels at the first lumbar puncture. It was reported that the kinetic difference in MBP release may be useful in the differential diagnosis of hemorrhagic and ischemic stroke. MBP levels in CSF also correlated to the visibility of the cerebral lesion at CT scan and to the short-term outcome of the patients. Further, the concentration of MBP increased with the extent of brain lesion and high values indicated a poor short-term prognosis for the patient. See Strand, T. et al, previously cited.

S100 protein is another marker which may be taken as a useful marker for assessing neurologic damage and for determining the extent of brain damage and for determining the extent of brain lesions. Thus, it has been suggested for use as an aid in the diagnosis and assessment of brain lesions and neurological damage due to stroke. See Missler, U., Weismann, M., Friedrich, C. and Kaps, M., S100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke, *Stroke* (1997) 28; 1956-60.

Neuron-specific enolase (NSE) also has been suggested as a useful marker of neurologic damage in the study of stroke with particular application in the assessment

of treatment. See Teasdale, G. and Jennett, B., Assessment of coma and impaired consciousness, Lancet (1974) 2; 81-84.

There continues to be a need for diagnostic techniques which can provide timely information concerning the type of stroke suffered by a patient, the onset of occurrence, the location of the event, the identification of appropriate patients who will benefit from treatment with the appropriate drug and the identification of patients who are at risk of bleeding as a result of treatment. Such techniques can provide data which will allow a physician to determine quickly the appropriate treatment required by the patient and permit early intervention.

It is therefore an object of this invention to provide a method for rapidly diagnosing and distinguishing stroke.

It is a further object of the invention to provide a method for distinguishing between thrombotic strokes and hemorrhagic strokes.

It is another object of the invention to provide such a method which includes analyzing the body fluid of a patient for at least four markers of stroke.

It is yet another object to provide a method which can provide information relating to the time of onset of the stroke.

It is still another object to provide diagnostic assay devices for use in the method.

SUMMARY OF THE INVENTION

These and other objects and advantages are accomplished in accordance with the invention by providing a method that is capable of determining whether a patient has suffered a stroke and, if so, whether the event is thrombotic or hemorrhagic. According to the method, a body fluid of the patient is analyzed for four molecules which are cell type specific, three of which are specific ischemic markers, namely S100 protein, myelin basic protein (MBP) and specific neuronal enolase (NSE) and one brain endothelial membrane protein, for example, thrombomodulin (Tm). The method analyzes the isoforms of the marker proteins which are specific to the brain.

The analyses of these markers may be carried out on the same sample of body fluid or on multiple samples of body fluid. In the latter embodiment the different body fluid samples may be taken at the same time or at different time periods.

5 The information which is obtained according to the method of the invention can be provided at the critically important early stages of a stroke, e.g., within the first three to six hours after onset of symptoms since the analysis of the patient's body fluid can be carried out in about 45 to 50 minutes after the body fluid is collected. The data can be vital to the physician by assisting in the
10 determination of how to treat a patient presenting with symptoms of stroke or suspected of having a stroke. The data can rule stroke in or out, and differentiate between ischemic and hemorrhagic stroke and therefore exclude hemorrhagic stroke patients from being given clot dissolving therapeutics because of the risk of increased bleeding. The data can also identify patients who are at risk of bleeding
15 as a result of treatment, i.e., patients with compromised brain vasculature. Further, the method can provide at an early stage prognostic information relating to the outcome of intervention which can improve patient selection for appropriate therapeutics and intervention. The method of the invention is diagnostic well before the imaging technologies. In addition, these data can indicate the location
20 of the stroke within the brain and the extent of damage to the brain as well as determine whether the extent of the stroke is increasing. The cerebral infarct associated with stroke, made up of dead and dying brain tissue, which forms because of inadequate oxygenation typically increases in size during the acute period after ischemia begins. By measuring the markers in samples of body fluid
25 taken at different points in time the progress of the stroke can be ascertained.

 According to a further broad aspect of the present invention there is provided a method for diagnosing and distinguishing stroke. The method comprises analyzing the body fluid of a patient to detect the presence and
30 concentration of four markers of stroke and wherein a first marker is myelin basic protein, a second marker is the beta isoform of S100 protein, a third marker is neuronal specific enolase, and a fourth marker is a brain endothelial cell

membrane protein. From the information obtained from the analyses one verifies whether an ischemic or hemorrhagic cerebral event has occurred and differentiates a particular type of cerebral event.

According to a further broad aspect of the present invention there is
5 provided a diagnostic kit for diagnosing and distinguishing stroke and which comprises at least four antibodies which are specific for each of four different marker proteins, and wherein the antibodies immobilized on a solid support. A first marker protein is myelin basic protein and a first antibody is specific therefor. A second marker protein is the beta isoform of S100 protein and a second
10 antibody is specific therefor. A third marker protein is neuronal specific enolase and a third antibody is specific therefor. A fourth marker protein is a brain endothelial cell membrane protein and a fourth antibody is specific therefor and at least four labeled antibodies. Each of the labeled antibodies binds to one of the marker protein.

15 According to a still further broad aspect of the present invention there is provided a method for the differential diagnosis of ischemic and hemorrhagic cerebral events. The method comprises analyzing the body fluid of a patient to detect the presence and concentration level of one or more ischemic marker proteins selected from the group consisting of myelin basic protein, the beta
20 isoform of S100 protein, neuronal specific enolase and combinations thereof. The body fluid of the patient is analyzed to detect the presence and concentration level of a brain endothelial cell membrane protein. From the information obtained from the analyses, the occurrence of an ischemic or hemorrhagic cerebral event is verified, and differentiating a particular type of cerebral event.

25 BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described further in detail with respect to various preferred embodiments thereof in conjunction with the accompanying drawings wherein:

Fig. 1 is a graphical illustration of the concentration over time (in minutes) of two
30 marker proteins which are indicative of cerebral condition or status;

Fig. 2 is a flow chart illustrating how data obtained according to an embodiment of the invention can be used for the diagnosis of cerebral condition or status; and

Figs. 3-10 are graphical illustrations of the concentration over time (in days) of four marker proteins analyzed according to an embodiment of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The markers which are analyzed according to the method of the invention are released into the circulation and are present in the blood and other body fluids. Preferably blood, or any blood product that contains them such as, for example, plasma, serum, cytolyzed blood (e.g., by treatment with hypotonic buffer or detergents), and dilutions and preparations thereof is analyzed according to the invention. In another preferred embodiment the concentration of the markers in CSF is measured.

The terms "above normal" and "above threshold" are used herein to refer to a level of a marker that is greater than the level of the marker observed in normal individuals, that is, individuals who are not undergoing a cerebral event, i.e. an injury to the brain which may be ischemic, mechanical or infectious. For some markers, no or infinitesimally low levels of the marker may be present normally in an individual's blood. For others of the markers analyzed for according to the invention, detectable levels may be present normally in blood. Thus, these terms contemplate a level that is significantly above the normal level found in individuals. The term "significantly" refers to statistical significance and generally means a two standard deviation (SD) above normal, or higher, concentration of the marker is present. The assay method by which the analysis for any particular marker protein is carried out must be sufficiently sensitive to be able to detect the level of the marker which is present over the concentration range of interest and also must be highly specific.

The four primary markers which are measured according to the present method are proteins which are released by the specific brain cells as the cells become damaged during a cerebral event. These proteins can be either in their native form or immunologically detectable fragments of the proteins resulting, for example, by

enzyme activity from proteolytic breakdown. The specific four primary markers when mentioned in the present application, including the claims hereof, are intended to include fragments of the proteins which can be immunologically detected. By "immunologically detectable" is meant that the protein fragments contain an epitope
5 which is specifically recognized by a cognate antibody.

As mentioned previously, the markers analyzed according to the method of the invention are cell type specific. Myelin basic protein (MBP) is a highly basic protein, localized in the myelin sheath, and accounts for about 30% of the total protein of the myelin in the human brain. The protein exists as a single polypeptide chain of 170
10 amino acid residues which has a rod-like structure with dimensions of 1.5×150 nm and a molecular weight of about 18,500 Dalton. It is a flexible protein which exists in a random coil devoid of α helices β conformations.

The increase of MBP concentration in blood and CSF is most evident about four to five days after the onset of ischemic stroke while in cerebral hemorrhage the increase is highest almost immediately after the onset. Further, patients with TIA
15 have normal values for MBP while those with cerebral infarction and intercerebral hemorrhage have elevated values. A normal value for a person who has not had a cerebral event is from 0.00 to about 0.016 ng/mL. MBP has a half-life in serum of about one hour and is a sensitive marker for cerebral hemorrhage.

20 The S100 protein is a cytoplasmic acidic calcium binding protein found predominantly in the gray matter of the brain, primarily in glia and Schwann cells. The protein exists in several homo- or heterodimeric isoforms consisting of two immunologically distinct subunits, alpha (MW = 10,400 Dalton) and beta (MW = 10,500 Dalton) while the S100 $\alpha\alpha$ is the homodimer $\alpha\alpha$ which is found mainly in
25 striated muscle, heart and kidney. The S100b isoform is the 21,000 Dalton homodimer $\beta\beta$. It is present in high concentration in glial cells and Schwann cells and is thus tissue specific. It is released during acute damage to the central nervous system and is a sensitive marker for cerebral infarction. According to the method of the invention, the assay is specific for the β -subunit of the S100 protein.

The S100b isoform is a specific brain marker released during acute damage to the central nervous system. It is eliminated by the kidney and has a half-life of about two hours in human serum. Repeated measurements of S100 serum levels are useful to follow the course of neurologic damage. Additionally, the presence of elevated
 5 S100 levels in CSF or serum, in association with stroke symptoms, can be useful in the differential diagnosis of stroke and may be a valuable indicator of cerebral infarction.

The enzyme enolase (EC 4.2.1.11) catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway. The enzyme
 10 exists in three isoproteins each the product of a separate gene. The gene loci have been designated ENO1, ENO2 and ENO3. The gene product of ENO1 is the nonneuronal enolase (NNE or α), which is widely distributed in various mammalian tissues. The gene product of ENO2 is the muscle specific enolase (MSE or β) which is localized mainly in the cardiac and striated muscle, while the product of the ENO3
 15 gene is the neuronal specific enolase (NSE or γ) which is largely found in the neurons and neuroendocrine cells. The native enzymes are found as homo- or heterodimeric isoforms composed of three immunologically distinct subunits, α , β and γ . Each subunit has a molecular weight of approximately 39,000 Dalton.

The $\alpha\gamma$ and $\gamma\gamma$ enolase isoforms, which have been designated neuronal specific
 20 enolase (NSE) each have a molecular weight of approximately 80,000 Dalton. It has been shown that NSE concentration in CSF increases after experimental focal ischemia and the release of NSE from damaged cerebral tissue into the CSF reflects the development and size of the infarcts. NSE has a serum half-life of about 48 hours and its peak concentration has been shown to occur later after cerebral artery (MCA)
 25 occlusion. NSE levels in CSF have been found to be elevated in acute and/or extensive disorders including subarachnoid hemorrhage and acute cerebral infarction.

The fourth marker protein measured according to the invention is a brain endothelial membrane protein. Endothelial cells which line the small blood vessels of the brain possess a unique expression of cell surface, receptors, transporters and
 30 intracellular enzymes that serve to tightly regulate exchange of solutes between blood

and brain parenchyma. Brain endothelial membrane proteins include: Thrombomodulin (Tm), a 105,000 Dalton surface glycoprotein involved in the regulation of intravascular coagulation; Glucose Transporter, (Gluc 1), a 55,000 Dalton cell surface transmembrane protein which may exist in dimeric or tetrameric form; Neurothelin/HT7, a 43,000 Dalton protein integrated into the cytoplasmic membrane transport protein; Gamma Glutamyl Transpeptidase, a protein which is found as a heterodimeric isoform composed of 22,000 and 25,000 Dalton subunits and is involved in the transfer of gamma glutamyl residue from glutathione to amino acids; and P-glycoprotein, a multidrug resistant membrane spanning protein. In a preferred embodiment of the method Tm is the brain endothelial membrane protein which is measured. Tm is a sensitive marker for lacunar infarcts.

The data obtained according to the method indicate whether a stroke has occurred and, if so, the type of stroke, the localization of the damage and the spread of the damage. Where the levels of all four markers are negative, i.e., within the normal range, there is no cerebral injury. When only the brain endothelial membrane protein, e.g., Tm, is elevated, or positive, i.e., the level is at least 2SD above normal, the stroke is a lacunar infarct present in the basal ganglia and deep white matter of the brain. When the NSE level is positive and the S100 and/or MBP levels are negative (the brain endothelial membrane protein marker is positive or negative) the patient has suffered a TIA.

According to another preferred embodiment, a fifth marker, which is from the specific cell type of one of the three ischemic markers analyzed according to the method of the invention, is measured to provide information related to the time of onset of the stroke. It should be recognized that the onset of stroke symptoms is not always known, particularly if the patient is unconscious or elderly and a reliable clinical history is not always available. An indication of the time of onset of the stroke can be obtained by relying on the differing release kinetics of brain markers having different molecular weights. The time release of brain markers into the circulation following brain injury is dependent on the size of the marker, with smaller markers tending to be released earlier in the event while larger markers tend to be released later. Fig. 1 illustrates the release kinetics of two marker proteins which are

analyzed according to the method of the invention, namely MBP and S100. These data were obtained from fluid collected from the brain tissue of a pig after coronary bypass surgery was performed. The samples were collected at 0, 30, 120, 180 and 240 minutes after the subject had been removed from the bypass machine. The concentration values are expressed in multiples of a baseline value which was the concentration at time zero. These data indicate that the release of MBP (MW = 18,500) appears to reach a maximum about 120 minutes after the ischemic event whereas the release of S100 (MW = 21,000) does so at after about 180 minutes. Thus, by measuring an additional protein marker from the specific cell type of one of the three ischemic markers utilized in the method of the invention, data relating to the time of onset can be obtained. The time of onset is defined as the moment of onset of clinical symptoms of stroke. In this preferred embodiment the second marker protein is a larger, i.e., a higher molecular weight marker, than the primary marker of the same cell type.

15 The three ischemic markers utilized according to the invention and various other high molecular weight markers from the same specific cell type are shown in Table I.

TABLE I		
MARKER	SIZE (D)	SMALLEST FRAGMENT (D)
SPECIFIC GLIAL MARKERS:		
S100	21,000	10,500
Growth Associated Protein 43 (GAP-43)	43,000	43,000
Glutamine Synthetase (GS)	400,000	44,000
Glial Fibrillary Acid Protein (GFAP)	51,000	51,000
Glycine Transporter (GLYT1)	50-70,000	50-70,000
Glycine Transporter (GLYT2)	90-110,000	90-110,000
SPECIFIC NEURONAL MARKERS:		
Neuron Specific Enolase (NSE)	78,000	39,000
Neruon Specific Glycoprotein (GP50)	42,000	42,000
Calpain	80,000	55,000
Neurofibrillary Protein (NF)	68,000	68,000
Heat Shock Protein 72 (HSP-72)	72,000	72,000
Beta Amyloid Precursor Protein (beta APP)	250,000	125,000
SPECIFIC AXONAL MARKERS:		
Myelin Basic Protein (MBP)	18,500	18,500
Calbindin D-28K	28,000	28,000
Proteolipid Protein (PLP)	23-30,000	23-30,000
Myelin Associated Glycoprotein (MAG)	90-100,000	58,000
Neurofilament H (HFN)	200,000	200,000

In a preferred embodiment of the invention body fluid samples taken from a patient at different points in time are analyzed. Typically a first body fluid sample is taken from a patient upon presentation with symptoms of stroke and analyzed according to the invention. Subsequently, some period of time after presentation, for example, about two hours after presentation, a second body fluid sample is taken and analyzed according to the invention. Referring now to Fig. 2 there is seen a flow chart illustrating how the data obtained from four marker proteins analyzed according to the invention, in the embodiment illustrated NSE, S100, MBP and Tm, can be used to triage the patient. The data can be used to diagnose stroke, rule out stroke,

distinguish between thrombotic and hemorrhagic stroke, identify appropriate patients for thrombolytic treatment and determine how the stroke is evolving.

As stated previously, the level of each of the four specific markers in the patient's body fluid can be measured from one single sample or one or more individual markers can be measured in one sample and at least one marker measured in one or more additional samples. By "sample" is meant a volume of body fluid such as blood or CSF which is obtained at one point in time. Further, as will be discussed in detail below, all the markers can be measured with one assay device or by using a separate assay device for each marker in which case aliquots of the same fluid sample can be used or different fluid samples can be used. It is apparent that the analyses should be carried out within some short time frame after the sample is taken, e.g., within about one-half hour, so the data can be used to prescribe treatment as quickly as possible. It is preferred to measure each of the four markers in the same single sample, irrespective of whether the analyses are carried out in a single analytical device or in separate such devices so the level of each marker simultaneously present in a single sample can be used to provide meaningful data.

Generally speaking, the presence of each marker is determined using antibodies specific for each of the markers and detecting immunospecific binding of each antibody to its respective cognate marker. Any suitable immunoassay method may be utilized, including those which are commercially available, to determine the level of each of the specific markers measured according to the invention. Extensive discussion of the known immunoassay techniques is not required here since these are known to those of skill in the art. Typical suitable immunoassay techniques include sandwich enzyme-linked immunoassays (ELISA), radioimmunoassays (RIA), competitive binding assays, homogeneous assays, heterogeneous assays, etc. Various of the known immunoassay methods are reviewed in *Methods in Enzymology*, 70, pp. 30-70 and 166-198 (1980). Direct and indirect labels can be used in immunoassays. A direct label can be defined as an entity, which in its natural state, is visible either to the naked eye or with the aid of an optical filter and/or applied stimulation, e.g., ultraviolet light, to promote fluorescence. Examples of colored labels which can be used include metallic sol particles, gold sol particles, dye sol particles, dyed latex

particles or dyes encapsulated in liposomes. Other direct labels include radionuclides and fluorescent or luminescent moieties. Indirect labels such as enzymes can also be used according to the invention. Various enzymes are known for use as labels such as, for example, alkaline phosphatase, horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase and urease. For a detailed discussion of enzymes in immunoassays see Engvall, Enzyme Immunoassay ELISA and EMIT, Methods of Enzymology, 70, 419-439 (1980).

A preferred immunoassay method for use according to the invention is a double antibody technique for measuring the level of the marker proteins in the patient's body fluid. According to this method one of the antibodies is a "capture" antibody and the other is a "detector" antibody. The capture antibody is immobilized on a solid support which may be any of various types which are known in the art such as, for example, microtiter plate wells, beads, tubes and porous materials such as nylon, glass fibers and other polymeric materials. In this method, a solid support, e.g., microtiter plate wells, coated with a capture antibody, preferably monoclonal, raised against the particular marker protein of interest, constitutes the solid phase. Diluted patient body fluid, e.g., serum or plasma, typically about 25 μ l, standards and controls are added to separate solid supports and incubated. When the marker protein is present in the body fluid it is captured by the immobilized antibody which is specific for the protein. After incubation and washing, an anti-marker protein detector antibody, e.g., a polyclonal rabbit anti-marker protein antibody, is added to the solid support. The detector antibody binds to marker protein bound to the capture antibody to form a sandwich structure. After incubation and washing an anti-IgG antibody, e.g., a polyclonal goat anti-rabbit IgG antibody, labeled with an enzyme such as horseradish peroxidase (HRP) is added to the solid support. After incubation and washing a substrate for the enzyme is added to the solid support followed by incubation and the addition of an acid solution to stop the enzymatic reaction.

The degree of enzymatic activity of immobilized enzyme is determined by measuring the optical density of the oxidized enzymatic product on the solid support at the appropriate wavelength, e.g., 450 nm for HRP. The absorbance at the wavelength is proportional to the amount of marker protein in the fluid sample. A set

of marker protein standards is used to prepare a standard curve of absorbance vs. marker protein concentration. This method is preferred since test results can be provided in 45 to 50 minutes and the method is both sensitive over the concentration range of interest for each marker and is highly specific.

5 The assay methods used to measure the marker proteins should exhibit sufficient sensitivity to be able to measure each protein over a concentration range from normal values found in healthy persons to elevated levels, i.e., 2SD above normal and beyond. Of course, a normal value range of the marker proteins can be found by analyzing the body fluid of healthy persons. For the S100b isoform where
10 +2SD = 0.02 ng/mL the upper limit of the assay range is preferably about 5.0 ng/mL. For NSE where +2SD = 9.9 ng/mL the upper limit of the range is preferably about 60 ng/mL. For MBP, which has an elevated level cutoff value of 0.02 ng/mL, the upper limit of the assay range is preferably about 5.0 ng/mL and for Tm, which has an elevated level cutoff value of about 73 ng/mL, the assay range upper limit is
15 preferably about 500 ng/mL.

The assays can be carried out in various assay device formats including those described in United States Patents 4,906,439; 5,051,237 and 5,147,609 to PB Diagnostic Systems, Inc.

20 The assay devices used according to the invention can be arranged to provide a semiquantitative or a quantitative result. By the term "semiquantitative" is meant the ability to discriminate between a level which is above the elevated marker protein value, and a level which is not above that threshold.

25 The assays may be carried out in various formats including, as discussed previously, a microtiter plate format which is preferred for carrying out the assays in a batch mode. The assays may also be carried out in automated immunoassay analyzers which are well known in the art and which can carry out assays on a number of different samples. These automated analyzers include continuous/random access types. Examples of such systems are described in United States Patents 5,207,987 and 5,518,688 to PB Diagnostic Systems, Inc. Various automated analyzers that are
30 commercially available include the OPUS® and OPUS MAGNUM® analyzers.

Another assay format which can be used according to the invention is a rapid manual test which can be administered at the point-of-care at any location. Typically, such point-of-care assay devices will provide a result which is above or below a threshold value, i.e., a semiquantitative result as described previously.

5 It should be recognized also that the assay devices used according to the invention can be provided to carry out one single assay for a particular marker protein or to carry out a plurality of assays, from a single volume of body fluid, for a corresponding number of different marker proteins. A preferred assay device of the latter type is one which can provide a semiquantitative result for the four primary
10 marker proteins measured according to the invention, i.e., S100b, NSE, MBP and a brain endothelial marker protein, e.g., Tm. These device typically are adapted to provide a distinct visually detectable colored band at the location where the capture antibody for the particular marker protein is located when the concentration of the marker protein is above the threshold level. For a detailed discussion of assay types
15 which can be utilized according to the invention as well as various assay formats and automated analyzer apparatus see U.S. Patent 5,747,274 to Jackowski.

The invention will now be described further in detail with respect to specific preferred embodiments, it being understood that these are intended to be illustrative only and the invention is not limited to the materials, procedures, etc. recited therein.

20

EXAMPLE

A prospective observational pilot study was carried out at two tertiary care hospitals. The study evaluated thirty three patients admitted with a clinical and computed tomographic (CT) diagnosis of acute ischemic stroke. The mean age of the patients presenting with stroke was approximately 66 years (66.4 ± 16.4) with an age
25 range of from 27 to 90 years. The mean delay between the onset of symptoms and presentation to the hospital was 22 hours with a range of from 1 to 72 hours. Admission National Institutes of Health Stroke Scale and Discharge modified Rankin scale scores were recorded. Blood samples were obtained on days 1 (presentation), 3, 5 and 7 at one hospital and days 1, 2 and 3 at the second hospital. All blood samples

were centrifuged and aliquots of serum were frozen and stored at -80°C until analysis for S100, NSE, MBP and Tm.

Control subjects included one hundred three healthy blood donors (age range from 18 to 78 years; mean age 54.6 ± 15.2 years) whose blood samples were used to determine reference values for concentrations of S100 and NSE and twenty four healthy blood donors who provided samples for reference measurements of MBP and Tm concentrations.

All reference values are reported as mean $+2\text{SD}$ unless otherwise stated. The reference value for S100 in serum was 0.0067 ng/mL with a 98th percentile of 0.020 ng/mL . An elevated S100 value was taken as any concentration greater than the 98th percentile (0.02 ng/mL) of normal (normal $+2\text{SD} = 0.02 \text{ ng/mL}$).

The reference value for NSE in serum was $5.03 \pm 2.40 \text{ ng/mL}$. An elevated NSE value was any concentration greater than 2SD above normal, 9.85 ng/mL .

The reference value for MBP in serum was $0.0162 \pm 0.0019 \text{ ng/mL}$. An elevated MBP value was any concentration greater than 2SD above normal, 0.02 ng/mL .

The reference value for Tm in serum was $50.52 \pm 13.62 \text{ ng/mL}$. An elevated Tm value was any concentration greater than $+2\text{SD}$ above normal, 76.14 ng/mL .

The levels of S100 and NSE were analyzed using Exact S100 and Exact NSE Elisa Assay Kits, respectively, available from Skye PharmaTech Inc., Mississauga, Canada. The levels of Tm were analyzed with an ELISA assay available from Diagnostica Stago, 9 rue des Freres Chausson, 92600 Asnieres Sur Seine, France. The level of MBP concentration was analyzed with an ELISA immunoassay from Diagnostic Systems Laboratories, Webster, Texas, United States.

In the tables showing the data obtained "D1" indicates the first day with the first blood sample being taken at the time of presentation. Subsequent days of sample collection are indicated by D2, D3, etc. For the values of the concentrations of the markers, $+2\text{SD}$ are above the normal range. "ND" signifies that no data was obtained.

TABLE II
NSE, S100, MBP ND T_m CONCENTRATIONS IN
CLINICAL SERUM SAMPLES

TABLE II						
NSE, S100, MBP ND T _m CONCENTRATIONS IN CLINICAL SERUM SAMPLES						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	T _m (ng/mL) + 2SD=73
SM-1 D1	42	Female	8.342	0.028	0.000	43.535
SM-1 D3			13.300	1.098	ND	61.946
SM-1 D5			9.622	0.060	0.238	65.859
SM-1 D7			10.710	0.066	1.725	62.177
DIAGNOSIS		Left internal carotid. CEREBRAL INFARCT (arteroembolic). 5h from onset of symptoms.				
OUTCOME		GOOD. Mild aphasia.				
SM-2 D1	55	Female	9.420	0.053	0.032	ND
SM-2 D3			5.430	0.015	0.105	ND
SM-2 D5			7.360	0.011	0.341	ND
SM-2 D7			9.906	0.008	0.124	ND
DIAGNOSIS		CEREBRAL INFARCT. Posterior circulation infarction (unknown mechanism). 20 h from onset of symptoms.				
OUTCOME		MODERATE. Dysarthria and hemiparesis.				
SM-3 D1	78	Male	12.670	0.112	0.000	92.324
SM-3 D3			14.980	0.719	1.420	101.990
SM-3 D5			28.570	1.301	4.845	119.251
DIAGNOSIS		CEREBRAL INFARCT. Total anterior circulation infarction (cardioembolic).				
OUTCOME		DEATH				
SM-4 D1	58	Male	8.520	0.008	0.000	73.913
SM-4 D3			4.406	0.028	0.147	78.286
SM-4 D5			4.888	0.024	0.265	85.881
DIAGNOSIS		CEREBRAL INFARCT. Lacunar circulation infarction (lacune).				
OUTCOME		GOOD. Mild ataxic hemiparesis.				

TABLE II
NSE, S100, MBP ND T_m CONCENTRATIONS IN
CLINICAL SERUM SAMPLES

CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SM-5 D2	27	Male	9.139	0.099	2.301	59.415
SM-5 D3			5.492	0.000	0.090	53.892
SM-5 D5			11.730	0.079	7.682	68.850
SM-5 D7			11.540	0.018	10.382	68.620
DIAGNOSIS		CEREBRAL INFARCT (fibromuscular dysplasia). 48h from onset of symptoms.				
OUTCOME		MODERATE. Aphasia and hemiparesis.				
SM-6 D1	63	Male	7.029	0.000	0.000	56.883
SM-6 D3			6.455	0.020	0.000	75.985
DIAGNOSIS		CEREBRAL INFARCT (unknown mechanism). 22 h from onset of symptoms.				
OUTCOME		MODERATE				
SM-7 D1	64	Female	8.566	0.021	0.013	105.212
SM-7 D3			5.061	0.024	0.000	129.146
SM-7 D5			6.783	0.021	0.017	129.607
SM-7 D8			7.377	0.015	0.000	162.746
DIAGNOSIS		CEREBRAL INFARCT. Lacunar circulation infarction (lacune).				
OUTCOME		MODERATE. Hemiparetic.				
SM-8 D1	45	Male	15.740	0.053	0.009	37.092
SM-8 D3			21.010	0.112	0.082	35.711
DM-8 D5			15.060	0.095	0.112	38.703
DIAGNOSIS		CEREBRAL INFARCT (Right vertebral dissection).				
OUTCOME		GOOD. Minimal deficit.				

TABLE II NSE, S100, MBP ND Tm CONCENTRATIONS IN CLINICAL SERUM SAMPLES						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SM-9 D1	35	Male	11.530	0.015	0.101	ND
SM-9 D5			8.033	0.021	0.040	ND
SM-9 D7			7.336	0.002	0.000	ND
DIAGNOSIS		CEREBRAL INFARCT (unknown mechanism).				
OUTCOME		GOOD. Minimal deficit.				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-01 D1	83	MALE	6.803	0.091	0.000	185.760
SJ-01 D2			8.566	0.235	0.000	166.659
SJ-01 D3			8.689	1.143	0.000	209.234
DIAGNOSIS		CEREBRAL INFARCT (recurrent). ↑BP, renal insufficiency, MI				
OUTCOME		Severe impairment developed on second day.				
SJ-02 D1	61	MALE	14.040	0.054	0.433	476.193
SJ-02 D2			13.430	0.110	1.199	403.010
SJ-02 D3			12.890	0.247	2.625	501.739
DIAGNOSIS		CEREBRAL INFARCT (parietal infarction), renal failure, MI, CA. 48 h from onset of symptoms				
OUTCOME		First CT negative. Second CT positive (Day 3). DEATH (day 5)				
SJ-03 D1	83	MALE	10.700	0.000	0.000	75.064
SJ-03 D2			8.926	0.000	0.000	81.968
SJ-03 D3			9.000	0.000	0.000	89.793
DIAGNOSIS		CEREBRAL INFARCT (lacune). ↑BP, DM				
OUTCOME		CT positive (Day 2)				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-04 D1	70	FEMALE	10.270	0.000	0.000	134.209
DIAGNOSIS		TIA. ↑BP, DM				
OUTCOME						
SJ-05 D1	72	MALE	6.639	0.000	0.326	185.760
SJ-05 D2			10.870	0.000	0.219	136.281
SJ-05 D3			8.197	0.000	0.387	132.598
DIAGNOSIS		CEREBRAL INFARCT (lacune), renal impairment				
OUTCOME		First CT negative				
SJ-06 D1	81	FEMALE	10.440	0.001	0.086	ND
DIAGNOSIS		CEREBRAL INFARCT. Renal impairment (dialysis). 36 h from onset of symptoms				
OUTCOME						
SJ-07 D1	90	FEMALE	12.540	0.001	0.162	ND
DIAGNOSIS		CEREBRAL INFARCT. 36 h from onset of symptoms				
OUTCOME						
SJ-08 D1	81	MALE	12.450	0.749	0.017	82.198
DIAGNOSIS		HAEMORRHAGIC. 1 h from onset of symptoms				
OUTCOME		CT positive. DEATH 2 h later.				
SJ-09 D1	46	MALE	4.891	0.000	0.000	88.182
SJ-09 D2			3.913	0.000	0.000	87.722
SJ-09 D3			1.848	0.000	0.000	105.903
DIAGNOSIS		STROKE (clinically). PA within 3 h of onset of symptoms				
OUTCOME		CT negative				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-10 D1	69	FEMALE	8.303	0.000	0.000	79.437
SJ-10 D2			6.000	0.000	0.000	74.144
SJ-10 D3			3.939	0.000	0.000	68.850
DIAGNOSIS		~12 h from onset of symptoms - numbness in arms - R side facial droop; difficulty swallowing - no past Hx CVA - patient diabetic; has Hx high BP				
OUTCOME		Initial CT negative. All symptoms resolved; except patient still unable to swallow.				
SJ-11 D1	39	MALE	10.770	0.058	0.063	65.398
SJ-11 D2			12.050	0.047	0.128	69.311
SJ-11 D3			17.330	0.068	0.189	76.675
DIAGNOSIS		CEREBRAL INFARCT. ~24 h from onset of symptoms - found unconscious with R-sided neglect				
OUTCOME		CT positive (Day 1) - 3 lesions present ~2 cm - basal ganglia L side Patient still has severe weakness R side with speech impairment				
SJ-12 D1	51	FEMALE	11.700	0.000	0.067	286.100
SJ-12 D2			8.788	0.000	0.055	270.911
SJ-12 D3			11.800	0.002	0.124	226.264
DIAGNOSIS		CEREBRAL INFARCT (lacune). ~ 12 h from onset of symptoms - weakness L side, esp. L arm - facial droop and pronounced slurring of speech - Bell's Palsy L side - renal dialysis patient				
OUTCOME		CT positive (Day 1) - developed thrombocytopenia Day 2				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-13 D1	78	FEMALE	10.090	0.000	0.000	46.297
SJ-13 D2 (Haemolytic)			40.040	0.768	0.433	41.924
SJ-13 D3			4.667	0.103	0.000	36.861
DIAGNOSIS		CEREBRAL INFARCT (Left MCA CVA) + CAD, + Diabetic, Hx HTN, + family Hx CVA. ~ 19 h from onset of symptoms				
OUTCOME		Initial CT negative. Initial symptoms worsened over 48 h to R hemiplegia.				
SJ-14 D1	72	MALE	7.303	0.087	0.299	NC
SJ-14 D2			5.697	0.007	0.055	NC
DIAGNOSIS		CEREBRAL INFARCT (Left CVA). ~ 9 h from onset of symptoms - prior CVA 1989 - Hx strial fib., anticoagulated - MI 1997				
OUTCOME		Symptoms improving				
SJ-15 D1	79	MALE	5.667	0.000	0.013	ND
DIAGNOSIS		CEREBRAL INFARCT (Left CVA) - symptoms progressive over 2 wk period; worsened over 3 day period just prior to presentation at hospital.				
OUTCOME		CT negative Day 1 - condition worsening at discharge (discharged at family's request for palliative care at home)				
SJ-16 D1	90	FEMALE	20.940	0.811	5.142	52.281
SJ-16 D2			12.220	0.498	5.459	55.733
SJ-16 D3			9.424	0.253	3.377	55.503
DIAGNOSIS		Large intracerebral bleed with smaller subdural hematoma and intraventricular hemorrhage - Onset of symptoms unknown (6 to 29 h prior) - previously well; no Hx other than colon Ca 20 yr prior; on no meds at home; found collapsed				
OUTCOME		Patient continues to worsen				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-17 D1	77	MALE	10.660	0.042	0.002	ND
SJ-17 D2			8.758	0.095	0.006	ND
SJ-17 D3			12.510	0.261	0.417	ND
DIAGNOSIS		CEREBRAL INFARCT (Right CVA) - old left cerebellar infarct - sudden onset; slurred speech and L-sided weakness ~ 15 h from onset of symptoms				
OUTCOME		CT showed old CVA and new right MCA infarct				
SJ-18 D1	79	MALE	21.560	0.008	0.000	61.946
SJ-18 D2			14.390	0.218	0.814	48.598
SJ-18 D3			11.050	0.102	0.698	55.963
DIAGNOSIS		Initial CT showed bleed or cerebral edema. ~ 2 h from onset of symptoms				
OUTCOME		Aphasia and R-sided weakness				
SJ-19 D1	82	FEMALE	9.948	0.000	ND	64.248
SJ-19 D2			9.781	0.008	ND	58.955
SJ-19 D3			11.720	0.023	ND	64.248
DIAGNOSIS		TIA ~ 24 h from onset of symptoms				
OUTCOME		Slurred speech, difficulty swallowing which persists.				
SJ-20 D1	ND	MALE	26.400	0.122	0.000	32.719
DIAGNOSIS		Haemorrhagic stroke				
OUTCOME						
SJ-21 D1	74	MALE	5.828	0.016	ND	74.374
SJ-21 D2			7.423	0.063	ND	75.985
SJ-21 D3			8.436	0.286	ND	71.382
DIAGNOSIS		CEREBRAL INFARCT (left CVA)				
OUTCOME		R-sided weakness				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-22 D1 (Haemolytic)	63	FEMALE	18.600	0.000	0.000	ND
SJ-22 D2			9.540	0.008	0.000	ND
DIAGNOSIS		CEREBRAL INFARCT (left CVA), initial CT negative				
OUTCOME		weakness (resolving)				
SJ-23 D1	79	MALE	14.530	2.009	5.478	ND
SJ-23 D2			23.980	>3.200	8.155	ND
SJ-23 D3			27.670	2.218	7.309	ND
DIAGNOSIS		CEREBRAL INFARCT, CT positive				
OUTCOME		CT showed multiple cerebral infarcts.				
SJ-24 D1	73	MALE	20.630	0.000	0.000	74.160
SJ-24 D2			17.880	0.000	0.000	89.750
SJ-24 D3			17.880	0.000	0.000	83.290
DIAGNOSIS		TIA - sudden decrease in ability to function, word difficulties				
OUTCOME		CT negative - Discharged with diagnosis of TIA				

The analysis of S100, NSE and MBP levels in serum samples from healthy control subjects showed no relationship of levels of these proteins to age or sex. In the case of Tm, the concentrations were higher in serum samples from healthy males than in females (54.62 ± 13.62 ng/mL, 2SD above normal = 81.86 ng/mL and 43.63 ± 11.18 ng/mL, 2SD above normal = 68.74 ng/mL, respectively).

Of the thirty three stroke patients twenty six were infarcts (79%) and of these five were lacunar (15%) and four had hemorrhagic stroke (12%). Of the hemorrhagic stroke patients three had subarachnoid hemorrhage and one had an intracerebral bleed. Three patients (9%) had transient ischemic attacks (TIA).

On presentation the levels of S100 were elevated in 44% of the patients, NSE levels were elevated in 59%, MBP levels were elevated in 40% and Tm levels were elevated in 57%.

5 The data indicate that by measuring the four marker proteins in accordance with the invention, where any one marker was elevated, 94% of the patients could be identified on presentation. Nineteen of the twenty one non-lacunar infarcts (90%) could be identified on presentation. The remaining two patients arrived at the hospital at 22 and 72 hours respectively after onset of symptoms.

10 Each of Figs. 3-10 is a graphical illustration of the data obtained from a different patient of the study. The concentration levels are expressed as multiples of a reference value and were obtained by dividing the actual measured concentration values by the defined reference value for each respective marker protein, i.e., the 2SD value.

15 All lacunar infarcts, hemorrhagic and TIA patients were identified on presentation with 100% accuracy. All five lacunar infarcts had elevated levels of Tm on presentation. In some patients the only elevated marker protein was Tm. Referring now to Fig. 3 it can be seen that, for patient SM7, the only elevated marker protein was Tm indicating a lacunar infarct.

20 The three TIA patients had elevated NSE levels and normal S100 and MBP levels that stayed within the normal range. Tm was elevated in one of the TIA patients. Referring now to Fig. 4 it can be seen that for patient SM-24, Tm was slightly elevated and NSE was elevated indicating a TIA. The patient was discharged with diagnosis of TIA. Referring now to Fig. 5 it can be seen that patient SM-3 had greatly elevated levels of MBP and S100 as well as elevated levels of NSE and Tm
25 indicating a cerebral infarct with damage spreading into the base of the brain.

In the four hemorrhagic stroke patients, the three subarachnoid hemorrhagic patients had elevated levels of S100 and NSE and a normal Tm level. In the patient with an intracerebral hemorrhagic stroke the levels of S100 and NSE were elevated and the level of MBP was elevated about 250 times. Fig. 6 illustrates that patient SJ-
30 16 had a 250 fold increased level of MBP upon presentation as well as elevated levels of S100 and NSE and had suffered an intracerebral hemorrhage.

Fig. 7 illustrates that patient SJ-2 had elevated MBP, Tm and S100 upon presentation and that the MBP and S100 levels continued to increase with time indicating a cerebral infarct with the stroke increasing over time. An initial CAT scan upon presentation was negative and became positive only days later.

5 Fig. 8 illustrates that patient SJ-18 presented with a TIA which evolved into a stroke. Tm was in the normal range indicating that the cerebral vasculature was not compromised and thus indicating that the patient was a good candidate for thrombolysis.

10 Fig. 9 illustrates that patient SM-8 presented with a cerebral infarct and, with Tm in the normal range, was a good candidate for thrombolysis since the endothelial vasculature was not compromised.

Fig. 10 illustrates that patient SJ-1 had a cerebral infarct and because of the elevated Tm level was at risk of hemorrhage if given thrombolytics because of the endothelial vasculature being compromised.

15 For the second serum sample obtained the levels of S100 were elevated in 73% of the stroke patients, the NSE levels in 54%, MBP levels in 64% and Tm levels in 55%. These data indicated that by measuring the four marker proteins in accordance with the invention, where any one marker was elevated 96% of the patients could be identified from the second serum sample obtained.

20 The data indicate that the levels of the protein markers in subsequent serum samples either increased or decreased depending upon whether the stroke was evolving in severity or subsiding.

25 Eighteen (54%) of the thirty three stroke patients had a CAT scan performed on presentation. All four hemorrhagic stroke patients were CAT positive at presentation. Nine (50%) of the eighteen patients had a normal CAT at presentation which became positive days later. Eight of these nine patients who had a normal CAT on presentation had elevated levels of one or more of the four protein markers on presentation. All of the nine CAT positive patients on presentation also had elevated levels of one or more protein markers on presentation.

Peak S100, NSE and MBP levels were significantly correlated (Pearson's) with admission NIHSS scores ($p < 0.05$) and discharge modified Rankin scores ($p < 0.05$).

5 The data show that levels of S100, NSE, MBP and Tm can be easily and reliably measured in acute ischemic stroke patients and that by measuring these four marker proteins in accordance with the invention, when any one marker protein is elevated a 94% sensitivity for acute ischemic stroke can be achieved upon presentation. Further, in the hyperacute period of the evolving stroke, elevated levels of one or more of these four marker proteins appear to precede irreversible tissue
10 damage and brain edema prior to detection of such damage by CAT.

Although the invention has been described with respect to various preferred embodiments it is not intended to be limited thereto but rather those skilled in the art will recognize that variations and modifications may be made therein which are within the spirit of the invention and the scope of the appended claims.

15

CLAIMS:

1. A method for diagnosing and distinguishing stroke comprising
 - a. analyzing the body fluid of a patient to detect the presence and concentration of four markers of stroke wherein
 - i. A first marker is myelin basic protein,
 - 5 ii. a second marker is the beta isoform of S100 protein,
 - iii. a third marker is neuronal specific enolase,
 - iv. a fourth marker is a brain endothelial cell membrane protein
 - and
 - b. from the information obtained from said analyses verifying
 - 10 whether an ischemic or hemorrhagic cerebral event has occurred and differentiating a particular type of cerebral event.
2. A method as defined in claim 1 wherein said body fluid is selected from the group consisting of blood, blood products and cerebrospinal fluid.
3. A method as defined in claim 1 wherein each of said analyses is carried out on the same sample of body fluid.
4. A method as defined in claim 1 wherein at least one of said analyses is carried out on a first sample of body fluid and at least another of said analyses is carried out on a second sample of body fluid.
5. A method as defined in claim 4 wherein said first and said second samples of body fluid are taken at different time periods.
6. A method as defined in claim 1 wherein said brain endothelial cell membrane protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7, Gamma Glutamyl Transpeptidase, P-glycoprotein and combinations thereof.

7. A method as defined in claim 1 wherein at least one of said analyses comprises contacting said body fluid with an antibody which is specific for said marker.

8. A method as defined in claim 7 wherein at least one of said analyses is carried out with an enzyme-labeled immunoassay method.

9. A method as defined in claim 1 and further including the step of analyzing said body fluid for a fifth marker protein, wherein said fifth marker protein has the same specific cell type as one of said first, second or third markers and has a higher molecular weight than said first, second or third marker which has the same
5 specific cell type.

10. A method as defined in claim 9 wherein at least one of said analyses comprises contacting said body fluid with an antibody which is specific for said marker.

11. A method as defined in claim 10 wherein at least one of said analyses is carried out with an enzyme-labeled immunoassay method.

12. A method as defined in claim 1 and further including the step of analyzing a second sample of a body fluid from said patient for said four markers, said second sample of body fluid being taken at a time subsequent to said body fluid analyzed in step a.

13. A method as defined in claim 1 wherein said steps of verifying and differentiating include comparing the concentration level detected in said analysis for each said four markers to a predefined threshold level for each said marker.

14. A diagnostic kit for diagnosing and distinguishing stroke comprising at least four antibodies which are specific for each of four different marker proteins, said antibodies immobilized on a solid support, wherein

- 10 a. a first marker protein is myelin basic protein and a first antibody is specific therefor,
- b. a second marker protein is the beta isoform of S100 protein and a second antibody is specific therefor,
- c. a third marker protein is neuronal specific enolase and a third
15 antibody is specific therefor, and
- d. a fourth marker protein is a brain endothelial cell membrane protein and a fourth antibody is specific therefor and at least four labeled antibodies, each of said labeled antibodies binding to one of said marker proteins.

15. A diagnostic kit as defined in claim 14 wherein each of said four antibodies is immobilized on the same solid support.

16. A diagnostic kit as defined in claim 14 wherein at least one of said four antibodies is immobilized on a first solid support and at least another of said four antibodies is immobilized on a second solid support.

17. A diagnostic kit as defined in claim 14 wherein at least one of said labeled antibodies comprises an enzyme-labeled antibody.

18. A diagnostic kit as defined in claim 14 wherein said brain endothelial cell marker protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7, Gamma Glutamyl Transpeptidase, P-glycoprotein and combinations thereof.

19. A diagnostic kit as defined in claim 14 and further including a fifth antibody which is specific for a fifth marker protein, wherein said fifth marker protein has the same specific cell type as one of said first, second or third markers and has a higher molecular weight than said first, second or third marker which has the same

5 specific cell type, and a fifth labeled antibody which binds to said fifth marker protein.

20. A diagnostic kit as defined in claim 19 wherein said fifth labeled antibody comprises an enzyme-labeled antibody.

21. A method for the differential diagnosis of ischemic and hemorrhagic
5 cerebral events comprising

a. analyzing the body fluid of a patient to detect the presence and concentration level of one or more ischemic marker proteins selected from the group consisting of myelin basic protein, the beta isoform of S100 protein, neuronal specific enolase and combinations thereof,

10 b. analyzing the body fluid of said patient to detect the presence and concentration level of a brain endothelial cell membrane protein, and

c. from the information obtained from said analyses, verifying the occurrence of an ischemic or hemorrhagic cerebral event and differentiating a particular type of cerebral event.

15 22. A method as defined in claim 21 wherein said steps of verifying and differentiating include comparing the concentration levels detected in said analyses for said one or more ischemic marker proteins and for said brain endothelial cell membrane protein to a predefined threshold level for each said ischemic marker protein and for said brain endothelial cell membrane protein.

20 23. A method as defined in claim 21 wherein said body fluid is selected from the group consisting of blood, blood products and cerebrospinal fluid.

24. A method as defined in claim 21 wherein said brain endothelial cell membrane protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7. Gamma Glutamyl
25 Transpeptidase, P-glycoprotein and combinations thereof.

25. A method as defined in claim 24 wherein said brain endothelial cell membrane protein is Thrombomodulin.

26. A method as defined in claim 21 further including
30 analyzing said body fluid to detect the presence and concentration level of a
secondary marker protein having the same specific cell type as one of said myelin
basic protein, beta isoform of S100 protein or neuronal specific enolase whereby the
time of onset of a hemorrhagic or ischemic cerebral event can be determined.

27. A method as defined in claim 26 wherein said secondary marker protein
35 has a higher molecular weight than said myelin basic protein, beta isoform of S100
protein or neuronal which has the same specific cell type.

28. A method as defined in claim 21 wherein each of said analyses is carried
on the same sample of body fluid.

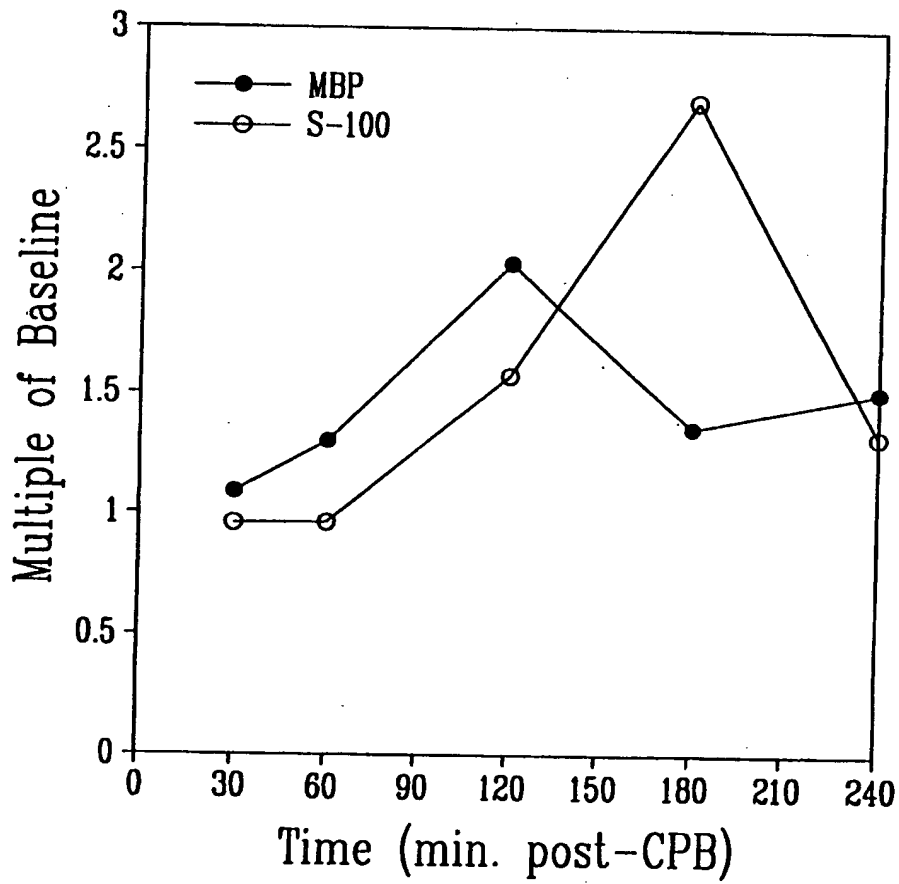
29. A method as defined in claim 21 wherein at least one of said analyses is
40 carried out on a first sample of body fluid and at least another of said analyses is
carried out on a second sample of body fluid.

30. A method as defined in claim 29 wherein said first and said second
samples of body fluid are taken at different time periods.

31. A method as defined in claim 21 wherein
45 a plurality of samples of said body fluid are obtained at predefined time
intervals and analyzed and the information from said analyses compared as a function
of time whereby the progression of an ischemic or hemorrhagic cerebral event can be
determined.

32. A method as defined in claim 21 wherein each of said analyses comprises
50 contacting said body fluid with an antibody which is specific for said protein.

33. A method as defined in claim 32 wherein at least one of said analyses is
carried out with an enzyme-labeled immunoassay method.

FIG - 1

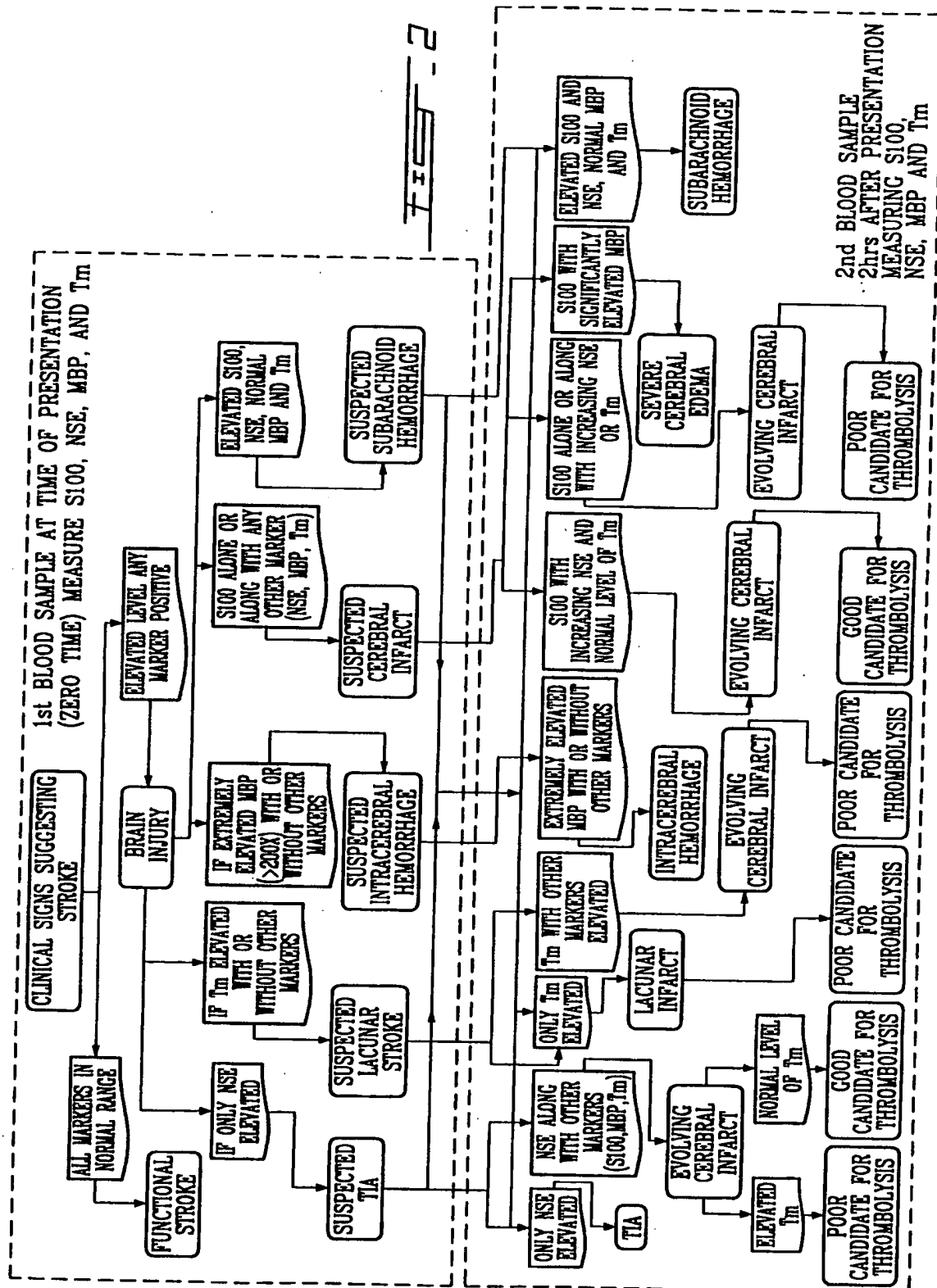


Fig. 2

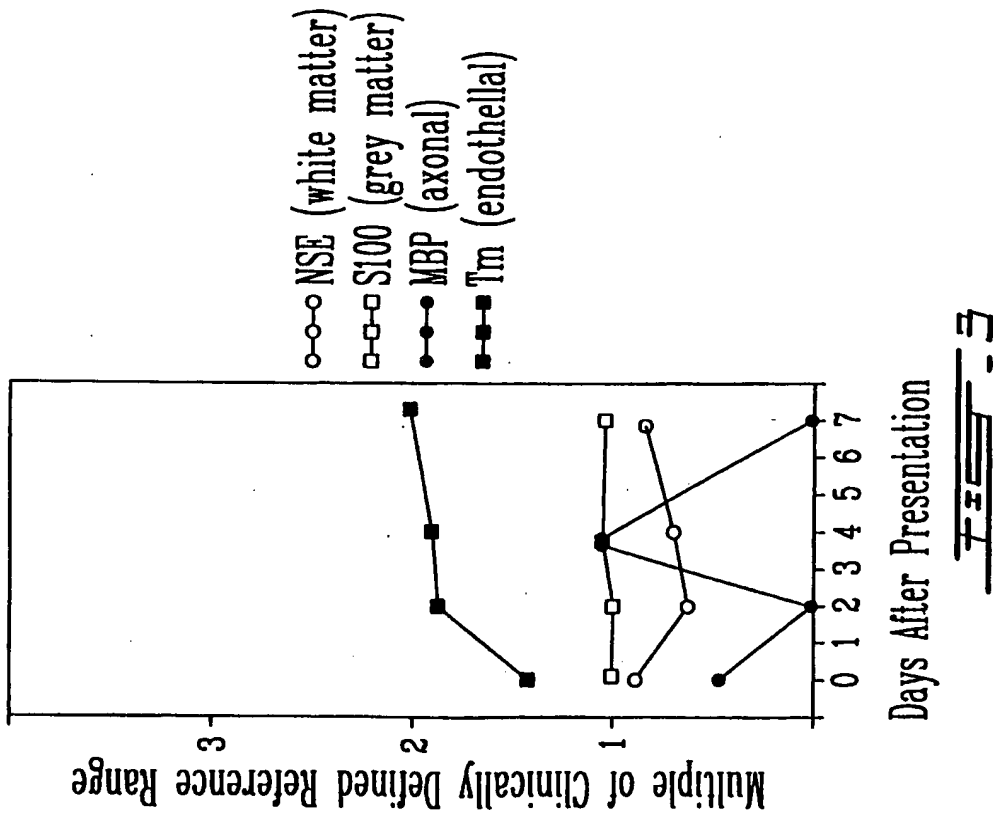


Figure 3

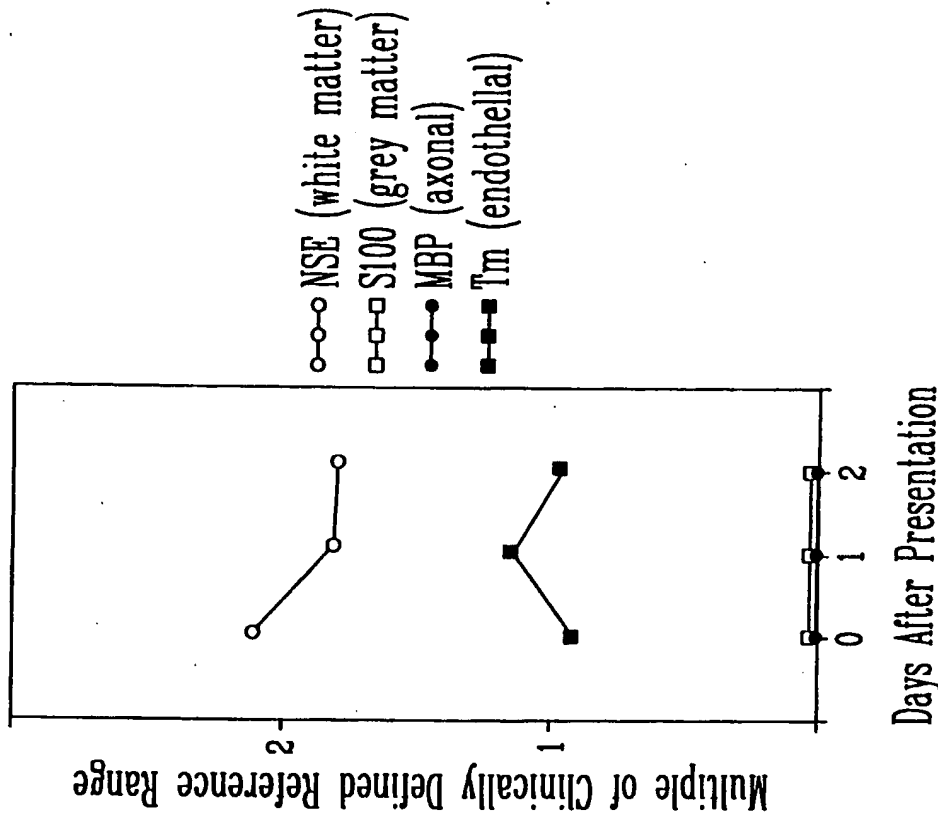
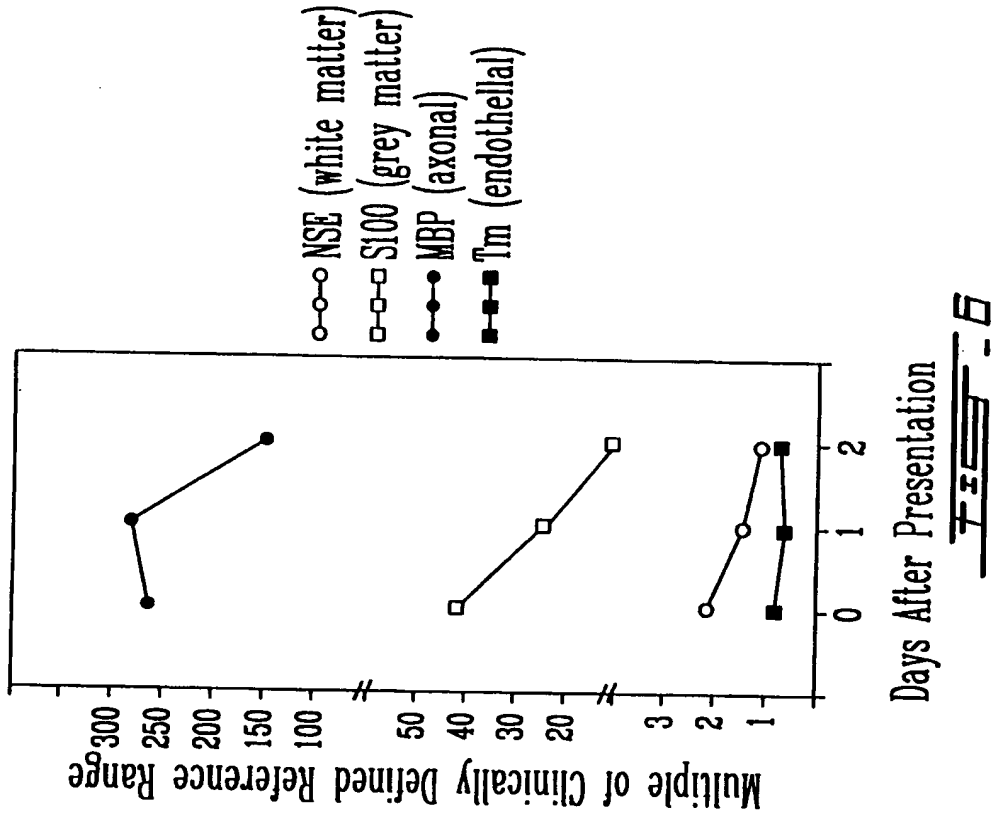
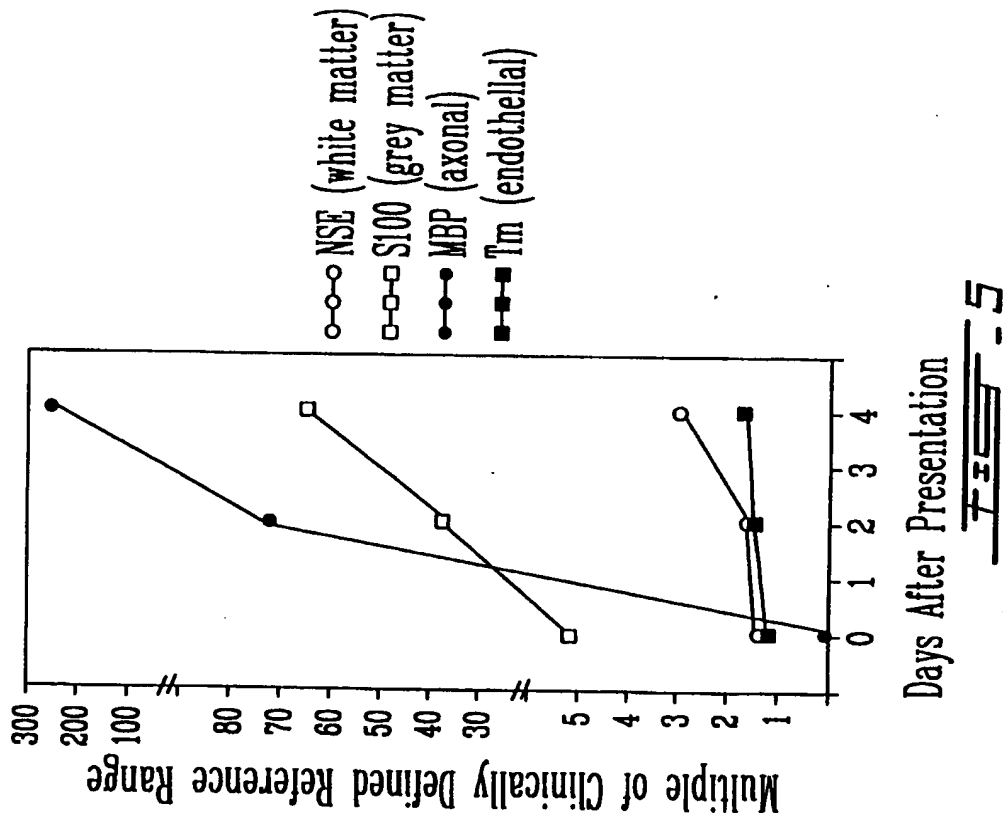
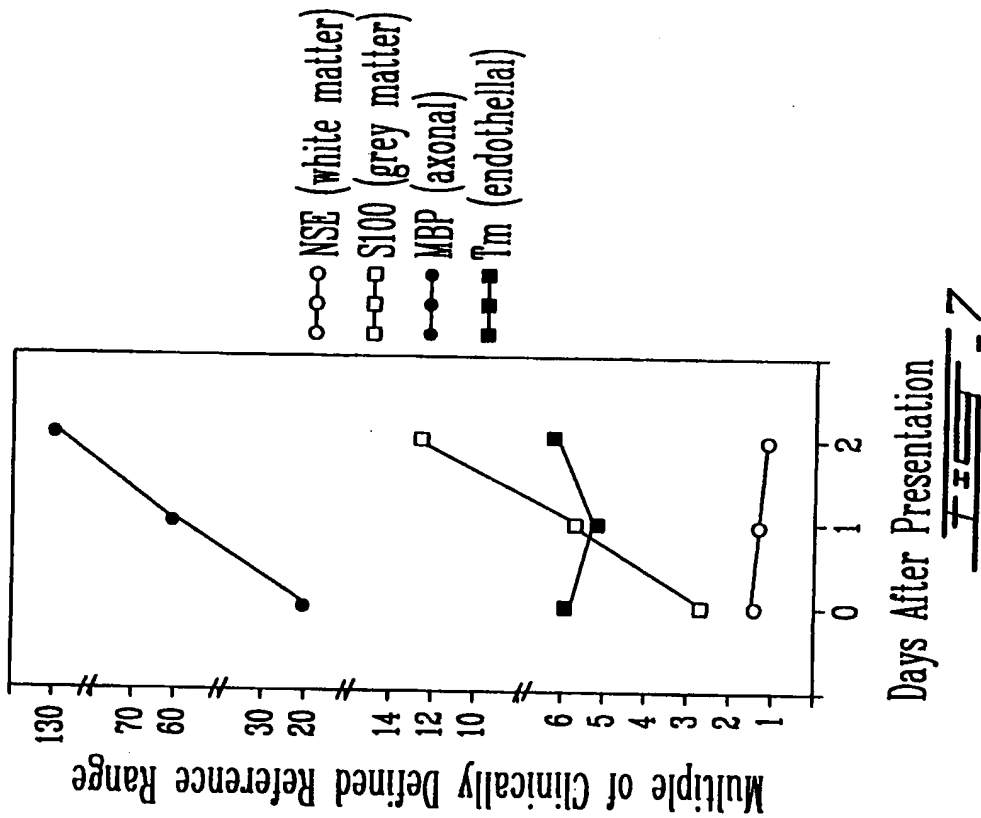
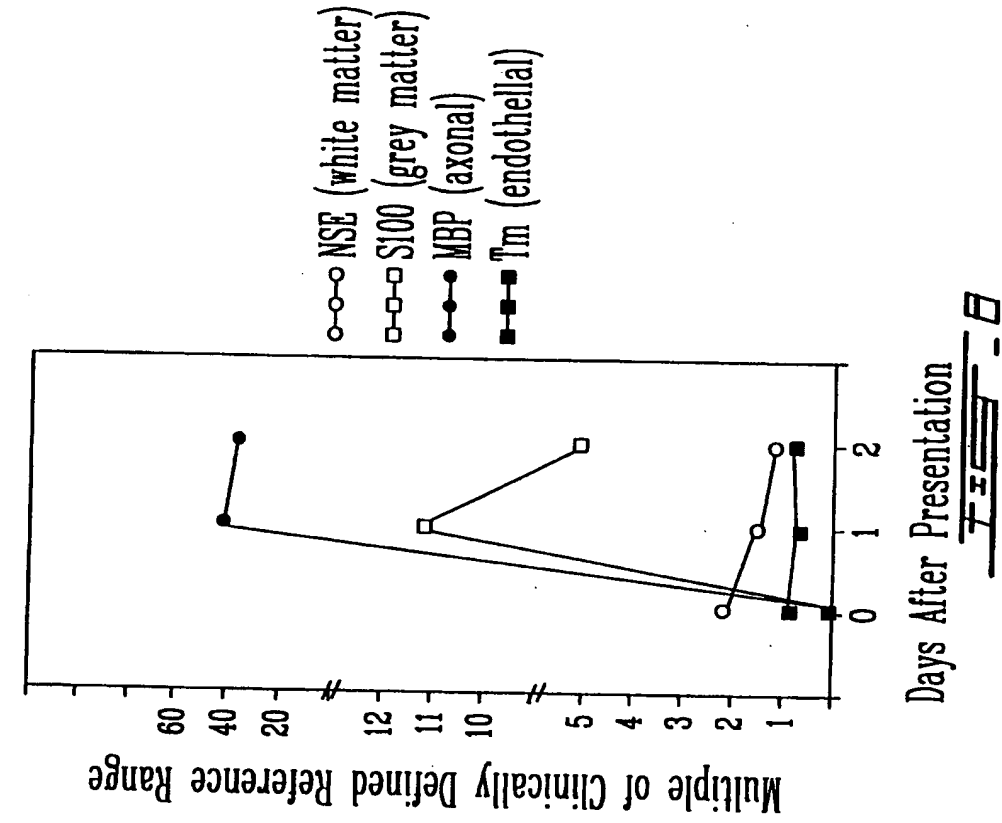
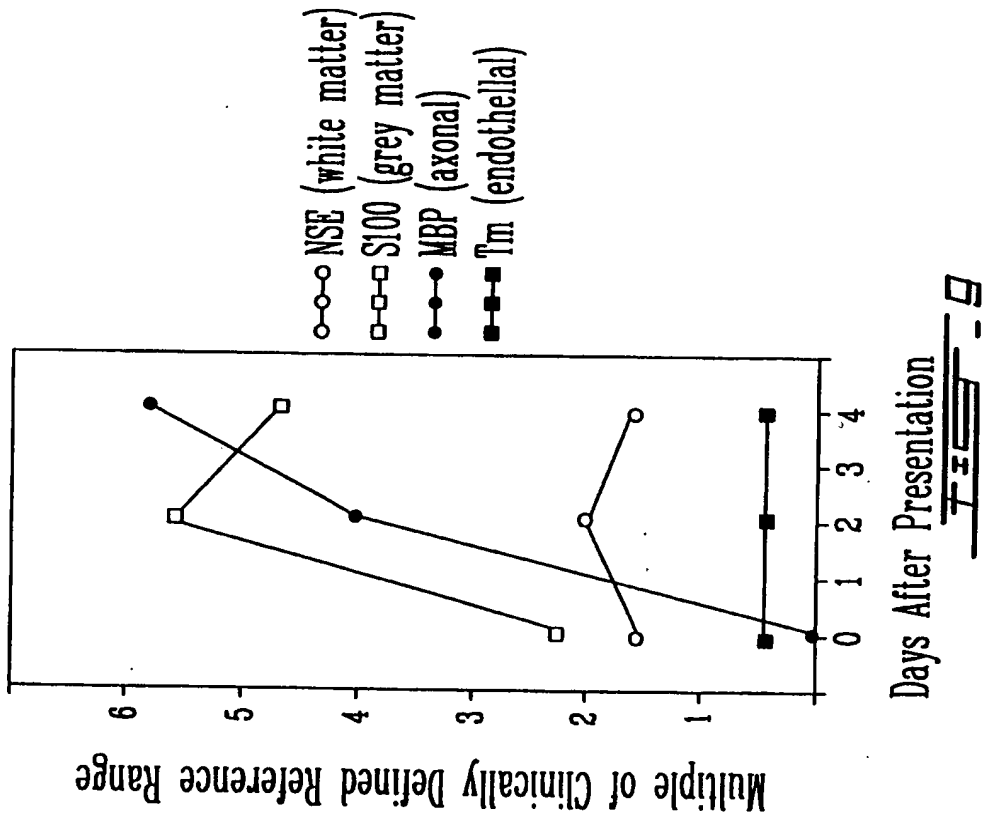
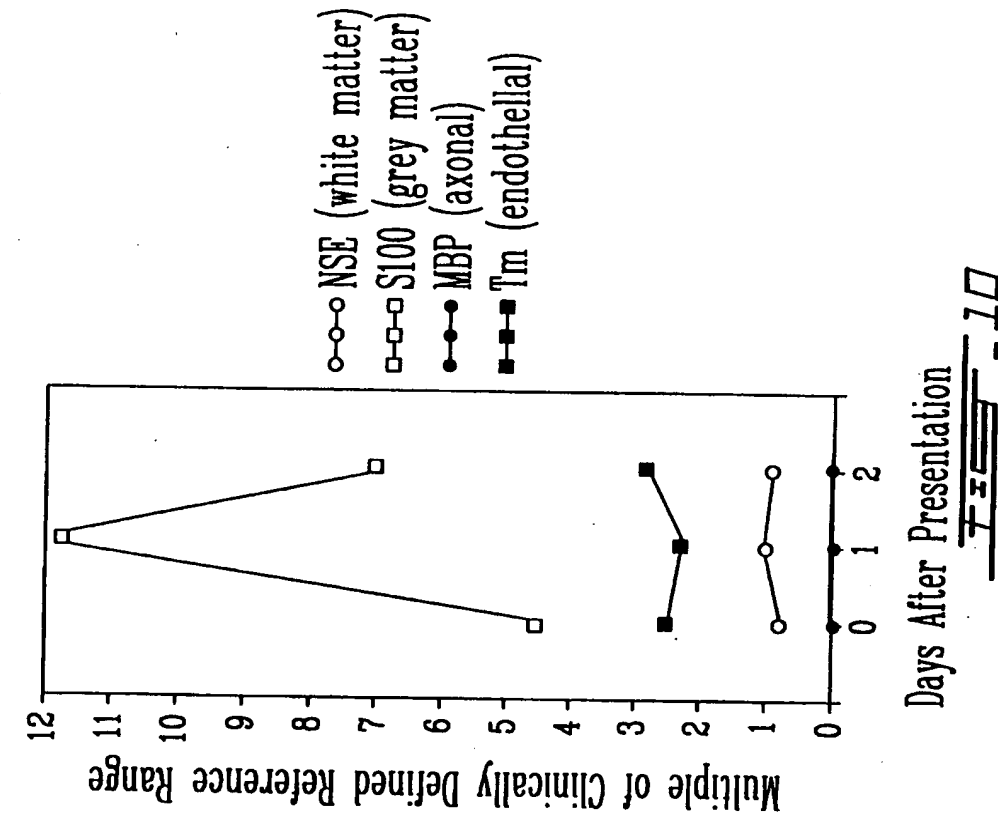
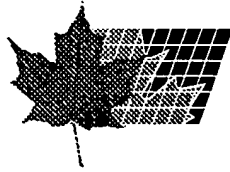


Figure 4



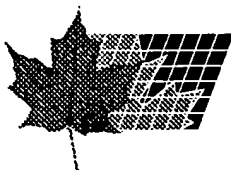






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(72) JENSEN, PETER H., DK
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(71) BEREZIN, VLADIMIR, DK
(51) Int. Cl.⁷ C07K 7/08, C07K 14/78, A61K 38/39, A61P 25/28
(30) 1998/09/29 (PA 1998 01232) DK
(30) 1999/04/29 (PA 1998 00592) DK
(54) **COMPOSES SE LIANT AUX N-CAM**
(54) **NCAM BINDING COMPOUNDS**

(57) The invention provides novel compounds which are capable to stimulate the proliferation or/and the outgrowth from cells presenting the neural cell adhesion molecule (NCAM). Additionally, the invention relates to pharmaceutical compositions, medicaments and methods for treatment of normal, degenerated and damaged NCAM presenting cells.



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C12Q 1/527

(54) **METHODE POUR DIAGNOSTIQUER ET DISTINGUER LES
ACCIDENTS VASCULAIRES CEREBRAUX ET DISPOSITIFS
DE DIAGNOSTIC UTILISES A CETTE FIN**

(54) **METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
AND DIAGNOSTIC DEVICES FOR USE THEREIN**

(57) A method for determining whether a subject has had a stroke and, if so, the type of stroke which includes analyzing the subject's body fluid for at least four selected markers of stroke, namely, myelin basic protein, S100 protein, neuronal specific enolase and a brain endothelial membrane protein such as thrombomodulin or a similar molecule. The data obtained from the analyses provide information as to the type of stroke, the onset of occurrence and the extent of brain damage and allow a physician to determine quickly the type of treatment required by the subject.

**METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
AND DIAGNOSTIC DEVICES FOR USE THEREIN**

ABSTRACT

A method for determining whether a subject has had a stroke and, if so, the type of stroke which includes analyzing the subject's body fluid for at least four
5 selected markers of stroke, namely, myelin basic protein, S100 protein, neuronal specific enolase and a brain endothelial membrane protein such as thrombomodulin or a similar molecule. The data obtained from the analyses provide information as to the type of stroke, the onset of occurrence and the extent of brain damage and allow a physician to determine quickly the type of treatment required by the subject.

**METHOD FOR DIAGNOSING AND DISTINGUISHING STROKE
AND DIAGNOSTIC DEVICES FOR USE THEREIN**

BACKGROUND OF THE INVENTION

5 This application is directed to a method for diagnosing whether a subject has had a stroke and, if so, differentiating between the different types of stroke. More specifically, the method includes analyzing the subject's body fluid for at least four selected markers of stroke. There are also described diagnostic devices and kits for use in the method.

10 The impact of stroke on the health of human beings is very great when considered in terms of mortality and even more devastating when disability is considered. For example, stroke is the third leading cause of death in adults in the United States, after ischemic heart disease and all forms of cancer. For people who survive, stroke is the leading cause of disability. The direct medical costs due to
15 stroke and the cost of lost employment amount to billions of dollars annually. Approximately 85% of all strokes are ischemic (thrombotic and embolic) with the remainder being hemorrhagic.

 Stroke is an underserved market for both therapeutics and diagnostic techniques. In the United States alone over 700,000 people have strokes each year. A
20 multiple of that number would be suspected of having strokes with diagnostics only confirmed by expensive technology including computer-assisted tomography (CAT) scans and magnetic resonance imaging (MRI). However, these sophisticated technologies are not available in all hospitals and they are also not sensitive enough to diagnose ischemic stroke at an early stage.

25 Stroke is a clinical diagnosis made by a neurologist, usually as a consultation. Current methods for diagnosing stroke include symptom evaluation, medical history, chest X-ray, ECG (electrical heart activity), EEG (brain nerve cell activity), CAT scan to assess brain damage and MRI to obtain internal body visuals. A number of blood tests may be performed to search for internal bleeding. These include complete blood

count, prothrombin time, partial thromboplastin time, serum electrolytes and blood glucose.

Determining the immediate cause of a stroke can be difficult especially upon presentation where the diagnosis relies mainly on imaging techniques. Approximately 50% of cerebral infarctions are not visible on a CAT scan. Further, even though a CAT scan can be very sensitive for the identification of hemorrhagic stroke, it is not very sensitive for cerebral ischemia during evaluation of stroke and is usually positive at from 24 to 36 hours after onset of stroke. As a result a window of opportunity for rapid treatment would usually have expired once the current diagnostic techniques positively identify a stroke.

The treatment of stroke includes preventive therapies such as antihypertensive and antiplatelet drugs which control and reduce blood pressure and thus reduce the likelihood of stroke. Also, the development of thrombolytic drugs such as t-PA (tissue plasminogen activator) has provided a significant advance in the treatment of ischemic stroke victims but to be effective and minimize damage from acute stroke it is necessary to begin treatment very early, for example, within about three hours after the onset of symptoms. These drugs dissolve blood vessel clots which block blood flow to the brain and which are the cause of approximately 80% of strokes. However, these drugs can also present the side effect of increased risk of bleeding. Various neuroprotectors such as calcium channel antagonist can stop damage to the brain as a result of ischemic insult. The window of treatment for these drugs is typically broader than that for the clot dissolvers and they do not increase the risk of bleeding.

Diagnostic techniques for the early diagnosis of stroke and identification of the type of stroke are needed to allow the physician to prescribe the appropriate therapeutic drugs at an early stage in the cerebral event. Various markers for stroke are known and analytical techniques for the determination of such markers have been described in the art. As used herein the term "marker" refers to a protein or other molecule that is released from the brain during a cerebral ischemic or hemorrhagic event. Such markers include isoforms of proteins that are unique to the brain.

It has been reported in the literature that myelin basic protein (MBP) concentration, in cerebrospinal fluid (CSF) increases after sufficient damage to

neuronal tissue, head trauma and AIDS dementia. Further, it has been reported that ultrastructural immunocytochemistry studies using anti-MBP antibodies have shown that MBP is localized exclusively in the myelin sheath. Thus, it has been suggested the MBP levels in CSF or serum be used as a marker of cerebral damage in acute cerebrovascular disease. See Strand, T., et al., Brain and plasma proteins in spinal fluid as markers for brain damage and severity of stroke, *Stroke* (1984) 15; 138-144. The increase in MBP concentration in CSF is most evident in about four to five days after the onset of thrombotic stroke while in cerebral hemorrhage the increase was highest almost immediately after onset. See Garcia-Alex, A., et al., Neuron-specific enolase and myelin basic protein: Relationship of cerebrospinal fluid concentration to the neurologic condition of asphyxiated full-term infants, *Pediatrics* (1994) 93; 234-240. It has also been found that patients with transitory ischemic attack (TIA) had normal CSF values for MBP while those with cerebral infarction and hemorrhage had elevated values. In cerebral infarction there was a significant increase in MBP concentration in CSF from the first to second lumbar puncture while patients with intracerebral hemorrhage had reached already markedly elevated levels at the first lumbar puncture. It was reported that the kinetic difference in MBP release may be useful in the differential diagnosis of hemorrhagic and ischemic stroke. MBP levels in CSF also correlated to the visibility of the cerebral lesion at CT scan and to the short-term outcome of the patients. Further, the concentration of MBP increased with the extent of brain lesion and high values indicated a poor short-term prognosis for the patient. See Strand, T. et al, previously cited.

S100 protein is another marker which may be taken as a useful marker for assessing neurologic damage and for determining the extent of brain damage and for determining the extent of brain lesions. Thus, it has been suggested for use as an aid in the diagnosis and assessment of brain lesions and neurological damage due to stroke. See Missler, U., Weismann, M., Friedrich, C. and Kaps, M., S100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke, *Stroke* (1997) 28; 1956-60.

Neuron-specific enolase (NSE) also has been suggested as a useful marker of neurologic damage in the study of stroke with particular application in the assessment

of treatment. See Teasdale, G. and Jennett, B., Assessment of coma and impaired consciousness, Lancet (1974) 2; 81-84.

There continues to be a need for diagnostic techniques which can provide timely information concerning the type of stroke suffered by a patient, the onset of occurrence, the location of the event, the identification of appropriate patients who will benefit from treatment with the appropriate drug and the identification of patients who are at risk of bleeding as a result of treatment. Such techniques can provide data which will allow a physician to determine quickly the appropriate treatment required by the patient and permit early intervention.

It is therefore an object of this invention to provide a method for rapidly diagnosing and distinguishing stroke.

It is a further object of the invention to provide a method for distinguishing between thrombotic strokes and hemorrhagic strokes.

It is another object of the invention to provide such a method which includes analyzing the body fluid of a patient for at least four markers of stroke.

It is yet another object to provide a method which can provide information relating to the time of onset of the stroke.

It is still another object to provide diagnostic assay devices for use in the method.

SUMMARY OF THE INVENTION

These and other objects and advantages are accomplished in accordance with the invention by providing a method that is capable of determining whether a patient has suffered a stroke and, if so, whether the event is thrombotic or hemorrhagic. According to the method, a body fluid of the patient is analyzed for four molecules which are cell type specific, three of which are specific ischemic markers, namely S100 protein, myelin basic protein (MBP) and specific neuronal enolase (NSE) and one brain endothelial membrane protein, for example, thrombomodulin (Tm). The method analyzes the isoforms of the marker proteins which are specific to the brain.

The analyses of these markers may be carried out on the same sample of body fluid or on multiple samples of body fluid. In the latter embodiment the different body fluid samples may be taken at the same time or at different time periods.

5 The information which is obtained according to the method of the invention can be provided at the critically important early stages of a stroke, e.g., within the first three to six hours after onset of symptoms since the analysis of the patient's body fluid can be carried out in about 45 to 50 minutes after the body fluid is collected. The data can be vital to the physician by assisting in the
10 determination of how to treat a patient presenting with symptoms of stroke or suspected of having a stroke. The data can rule stroke in or out, and differentiate between ischemic and hemorrhagic stroke and therefore exclude hemorrhagic stroke patients from being given clot dissolving therapeutics because of the risk of increased bleeding. The data can also identify patients who are at risk of bleeding
15 as a result of treatment, i.e., patients with compromised brain vasculature. Further, the method can provide at an early stage prognostic information relating to the outcome of intervention which can improve patient selection for appropriate therapeutics and intervention. The method of the invention is diagnostic well before the imaging technologies. In addition, these data can indicate the location
20 of the stroke within the brain and the extent of damage to the brain as well as determine whether the extent of the stroke is increasing. The cerebral infarct associated with stroke, made up of dead and dying brain tissue, which forms because of inadequate oxygenation typically increases in size during the acute period after ischemia begins. By measuring the markers in samples of body fluid
25 taken at different points in time the progress of the stroke can be ascertained.

According to a further broad aspect of the present invention there is provided a method for diagnosing and distinguishing stroke. The method comprises analyzing the body fluid of a patient to detect the presence and
30 concentration of four markers of stroke and wherein a first marker is myelin basic protein, a second marker is the beta isoform of S100 protein, a third marker is neuronal specific enolase, and a fourth marker is a brain endothelial cell

membrane protein. From the information obtained from the analyses one verifies whether an ischemic or hemorrhagic cerebral event has occurred and differentiates a particular type of cerebral event.

According to a further broad aspect of the present invention there is provided a diagnostic kit for diagnosing and distinguishing stroke and which comprises at least four antibodies which are specific for each of four different marker proteins, and wherein the antibodies immobilized on a solid support. A first marker protein is myelin basic protein and a first antibody is specific therefor. A second marker protein is the beta isoform of S100 protein and a second antibody is specific therefor. A third marker protein is neuronal specific enolase and a third antibody is specific therefor. A fourth marker protein is a brain endothelial cell membrane protein and a fourth antibody is specific therefor and at least four labeled antibodies. Each of the labeled antibodies binds to one of the marker protein.

According to a still further broad aspect of the present invention there is provided a method for the differential diagnosis of ischemic and hemorrhagic cerebral events. The method comprises analyzing the body fluid of a patient to detect the presence and concentration level of one or more ischemic marker proteins selected from the group consisting of myelin basic protein, the beta isoform of S100 protein, neuronal specific enolase and combinations thereof. The body fluid of the patient is analyzed to detect the presence and concentration level of a brain endothelial cell membrane protein. From the information obtained from the analyses, the occurrence of an ischemic or hemorrhagic cerebral event is verified, and differentiating a particular type of cerebral event.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described further in detail with respect to various preferred embodiments thereof in conjunction with the accompanying drawings wherein:

Fig. 1 is a graphical illustration of the concentration over time (in minutes) of two marker proteins which are indicative of cerebral condition or status;

Fig. 2 is a flow chart illustrating how data obtained according to an embodiment of the invention can be used for the diagnosis of cerebral condition or status; and

Figs. 3-10 are graphical illustrations of the concentration over time (in days) of four marker proteins analyzed according to an embodiment of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The markers which are analyzed according to the method of the invention are released into the circulation and are present in the blood and other body fluids. Preferably blood, or any blood product that contains them such as, for example, plasma, serum, cytolyzed blood (e.g., by treatment with hypotonic buffer or detergents), and dilutions and preparations thereof is analyzed according to the invention. In another preferred embodiment the concentration of the markers in CSF is measured.

The terms "above normal" and "above threshold" are used herein to refer to a level of a marker that is greater than the level of the marker observed in normal individuals, that is, individuals who are not undergoing a cerebral event, i.e. an injury to the brain which may be ischemic, mechanical or infectious. For some markers, no or infinitesimally low levels of the marker may be present normally in an individual's blood. For others of the markers analyzed for according to the invention, detectable levels may be present normally in blood. Thus, these terms contemplate a level that is significantly above the normal level found in individuals. The term "significantly" refers to statistical significance and generally means a two standard deviation (SD) above normal, or higher, concentration of the marker is present. The assay method by which the analysis for any particular marker protein is carried out must be sufficiently sensitive to be able to detect the level of the marker which is present over the concentration range of interest and also must be highly specific.

The four primary markers which are measured according to the present method are proteins which are released by the specific brain cells as the cells become damaged during a cerebral event. These proteins can be either in their native form or immunologically detectable fragments of the proteins resulting, for example, by

enzyme activity from proteolytic breakdown. The specific four primary markers when mentioned in the present application, including the claims hereof, are intended to include fragments of the proteins which can be immunologically detected. By "immunologically detectable" is meant that the protein fragments contain an epitope which is specifically recognized by a cognate antibody.

As mentioned previously, the markers analyzed according to the method of the invention are cell type specific. Myelin basic protein (MBP) is a highly basic protein, localized in the myelin sheath, and accounts for about 30% of the total protein of the myelin in the human brain. The protein exists as a single polypeptide chain of 170 amino acid residues which has a rod-like structure with dimensions of 1.5×150 nm and a molecular weight of about 18,500 Dalton. It is a flexible protein which exists in a random coil devoid of α helices β conformations.

The increase of MBP concentration in blood and CSF is most evident about four to five days after the onset of ischemic stroke while in cerebral hemorrhage the increase is highest almost immediately after the onset. Further, patients with TIA have normal values for MBP while those with cerebral infarction and intercerebral hemorrhage have elevated values. A normal value for a person who has not had a cerebral event is from 0.00 to about 0.016 ng/mL. MBP has a half-life in serum of about one hour and is a sensitive marker for cerebral hemorrhage.

The S100 protein is a cytoplasmic acidic calcium binding protein found predominantly in the gray matter of the brain, primarily in glia and Schwann cells. The protein exists in several homo- or heterodimeric isoforms consisting of two immunologically distinct subunits, alpha (MW = 10,400 Dalton) and beta (MW = 10,500 Dalton) while the S100 $\alpha\alpha$ is the homodimer $\alpha\alpha$ which is found mainly in striated muscle, heart and kidney. The S100b isoform is the 21,000 Dalton homodimer $\beta\beta$. It is present in high concentration in glial cells and Schwann cells and is thus tissue specific. It is released during acute damage to the central nervous system and is a sensitive marker for cerebral infarction. According to the method of the invention, the assay is specific for the β -subunit of the S100 protein.

The S100b isoform is a specific brain marker released during acute damage to the central nervous system. It is eliminated by the kidney and has a half-life of about two hours in human serum. Repeated measurements of S100 serum levels are useful to follow the course of neurologic damage. Additionally, the presence of elevated
5 S100 levels in CSF or serum, in association with stroke symptoms, can be useful in the differential diagnosis of stroke and may be a valuable indicator of cerebral infarction.

The enzyme enolase (EC 4.2.1.11) catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway. The enzyme
10 exists in three isoproteins each the product of a separate gene. The gene loci have been designated ENO1, ENO2 and ENO3. The gene product of ENO1 is the nonneuronal enolase (NNE or α), which is widely distributed in various mammalian tissues. The gene product of ENO2 is the muscle specific enolase (MSE or β) which is localized mainly in the cardiac and striated muscle, while the product of the ENO3
15 gene is the neuronal specific enolase (NSE or γ) which is largely found in the neurons and neuroendocrine cells. The native enzymes are found as homo- or heterodimeric isoforms composed of three immunologically distinct subunits, α , β and γ . Each subunit has a molecular weight of approximately 39,000 Dalton.

The $\alpha\gamma$ and $\gamma\gamma$ enolase isoforms, which have been designated neuronal specific
20 enolase (NSE) each have a molecular weight of approximately 80,000 Dalton. It has been shown that NSE concentration in CSF increases after experimental focal ischemia and the release of NSE from damaged cerebral tissue into the CSF reflects the development and size of the infarcts. NSE has a serum half-life of about 48 hours and its peak concentration has been shown to occur later after cerebral artery (MCA)
25 occlusion. NSE levels in CSF have been found to be elevated in acute and/or extensive disorders including subarachnoid hemorrhage and acute cerebral infarction.

The fourth marker protein measured according to the invention is a brain endothelial membrane protein. Endothelial cells which line the small blood vessels of the brain possess a unique expression of cell surface, receptors, transporters and
30 intracellular enzymes that serve to tightly regulate exchange of solutes between blood

and brain parenchyma. Brain endothelial membrane proteins include: Thrombomodulin (Tm), a 105,000 Dalton surface glycoprotein involved in the regulation of intravascular coagulation; Glucose Transporter, (Gluc 1), a 55,000 Dalton cell surface transmembrane protein which may exist in dimeric or tetrameric form; Neurothelin/HT7, a 43,000 Dalton protein integrated into the cytoplasmic membrane transport protein; Gamma Glutamyl Transpeptidase, a protein which is found as a heterodimeric isoform composed of 22,000 and 25,000 Dalton subunits and is involved in the transfer of gamma glutamyl residue from glutathione to amino acids; and P-glycoprotein, a multidrug resistant membrane spanning protein. In a preferred embodiment of the method Tm is the brain endothelial membrane protein which is measured. Tm is a sensitive marker for lacunar infarcts.

The data obtained according to the method indicate whether a stroke has occurred and, if so, the type of stroke, the localization of the damage and the spread of the damage. Where the levels of all four markers are negative, i.e., within the normal range, there is no cerebral injury. When only the brain endothelial membrane protein, e.g., Tm, is elevated, or positive, i.e., the level is at least 2SD above normal, the stroke is a lacunar infarct present in the basal ganglia and deep white matter of the brain. When the NSE level is positive and the S100 and/or MBP levels are negative (the brain endothelial membrane protein marker is positive or negative) the patient has suffered a TIA.

According to another preferred embodiment, a fifth marker, which is from the specific cell type of one of the three ischemic markers analyzed according to the method of the invention, is measured to provide information related to the time of onset of the stroke. It should be recognized that the onset of stroke symptoms is not always known, particularly if the patient is unconscious or elderly and a reliable clinical history is not always available. An indication of the time of onset of the stroke can be obtained by relying on the differing release kinetics of brain markers having different molecular weights. The time release of brain markers into the circulation following brain injury is dependent on the size of the marker, with smaller markers tending to be released earlier in the event while larger markers tend to be released later. Fig. 1 illustrates the release kinetics of two marker proteins which are

analyzed according to the method of the invention, namely MBP and S100. These data were obtained from fluid collected from the brain tissue of a pig after coronary bypass surgery was performed. The samples were collected at 0, 30, 120, 180 and 240 minutes after the subject had been removed from the bypass machine. The concentration values are expressed in multiples of a baseline value which was the concentration at time zero. These data indicate that the release of MBP (MW = 18,500) appears to reach a maximum about 120 minutes after the ischemic event whereas the release of S100 (MW = 21,000) does so at after about 180 minutes. Thus, by measuring an additional protein marker from the specific cell type of one of the three ischemic markers utilized in the method of the invention, data relating to the time of onset can be obtained. The time of onset is defined as the moment of onset of clinical symptoms of stroke. In this preferred embodiment the second marker protein is a larger, i.e., a higher molecular weight marker, than the primary marker of the same cell type.

15 The three ischemic markers utilized according to the invention and various other high molecular weight markers from the same specific cell type are shown in Table I.

TABLE I		
MARKER	SIZE (D)	SMALLEST FRAGMENT (D)
SPECIFIC GLIAL MARKERS:		
S100	21,000	10,500
Growth Associated Protein 43 (GAP-43)	43,000	43,000
Glutamine Synthetase (GS)	400,000	44,000
Glial Fibrillary Acid Protein (GFAP)	51,000	51,000
Glycine Transporter (GLYT1)	50-70,000	50-70,000
Glycine Transporter (GLYT2)	90-110,000	90-110,000
SPECIFIC NEURONAL MARKERS:		
Neuron Specific Enolase (NSE)	78,000	39,000
Neuron Specific Glycoprotein (GP50)	42,000	42,000
Calpain	80,000	55,000
Neurofibrillary Protein (NF)	68,000	68,000
Heat Shock Protein 72 (HSP-72)	72,000	72,000
Beta Amyloid Precursor Protein (beta APP)	250,000	125,000
SPECIFIC AXONAL MARKERS:		
Myelin Basic Protein (MBP)	18,500	18,500
Calbindin D-28K	28,000	28,000
Proteolipid Protein (PLP)	23-30,000	23-30,000
Myelin Associated Glycoprotein (MAG)	90-100,000	58,000
Neurofilament H (HFN)	200,000	200,000

In a preferred embodiment of the invention body fluid samples taken from a patient at different points in time are analyzed. Typically a first body fluid sample is taken from a patient upon presentation with symptoms of stroke and analyzed according to the invention. Subsequently, some period of time after presentation, for example, about two hours after presentation, a second body fluid sample is taken and analyzed according to the invention. Referring now to Fig. 2 there is seen a flow chart illustrating how the data obtained from four marker proteins analyzed according to the invention, in the embodiment illustrated NSE, S100, MBP and Tm, can be used to triage the patient. The data can be used to diagnose stroke, rule out stroke,

distinguish between thrombotic and hemorrhagic stroke, identify appropriate patients for thrombolytic treatment and determine how the stroke is evolving.

As stated previously, the level of each of the four specific markers in the patient's body fluid can be measured from one single sample or one or more individual markers can be measured in one sample and at least one marker measured in one or more additional samples. By "sample" is meant a volume of body fluid such as blood or CSF which is obtained at one point in time. Further, as will be discussed in detail below, all the markers can be measured with one assay device or by using a separate assay device for each marker in which case aliquots of the same fluid sample can be used or different fluid samples can be used. It is apparent that the analyses should be carried out within some short time frame after the sample is taken, e.g., within about one-half hour, so the data can be used to prescribe treatment as quickly as possible. It is preferred to measure each of the four markers in the same single sample, irrespective of whether the analyses are carried out in a single analytical device or in separate such devices so the level of each marker simultaneously present in a single sample can be used to provide meaningful data.

Generally speaking, the presence of each marker is determined using antibodies specific for each of the markers and detecting immunospecific binding of each antibody to its respective cognate marker. Any suitable immunoassay method may be utilized, including those which are commercially available, to determine the level of each of the specific markers measured according to the invention. Extensive discussion of the known immunoassay techniques is not required here since these are known to those of skill in the art. Typical suitable immunoassay techniques include sandwich enzyme-linked immunoassays (ELISA), radioimmunoassays (RIA), competitive binding assays, homogeneous assays, heterogeneous assays, etc. Various of the known immunoassay methods are reviewed in *Methods in Enzymology*, 70, pp. 30-70 and 166-198 (1980). Direct and indirect labels can be used in immunoassays. A direct label can be defined as an entity, which in its natural state, is visible either to the naked eye or with the aid of an optical filter and/or applied stimulation, e.g., ultraviolet light, to promote fluorescence. Examples of colored labels which can be used include metallic sol particles, gold sol particles, dye sol particles, dyed latex

particles or dyes encapsulated in liposomes. Other direct labels include radionuclides and fluorescent or luminescent moieties. Indirect labels such as enzymes can also be used according to the invention. Various enzymes are known for use as labels such as, for example, alkaline phosphatase, horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase and urease. For a detailed discussion of enzymes in immunoassays see Engvall, Enzyme Immunoassay ELISA and EMIT, Methods of Enzymology, 70, 419-439 (1980).

A preferred immunoassay method for use according to the invention is a double antibody technique for measuring the level of the marker proteins in the patient's body fluid. According to this method one of the antibodies is a "capture" antibody and the other is a "detector" antibody. The capture antibody is immobilized on a solid support which may be any of various types which are known in the art such as, for example, microtiter plate wells, beads, tubes and porous materials such as nylon, glass fibers and other polymeric materials. In this method, a solid support, e.g., microtiter plate wells, coated with a capture antibody, preferably monoclonal, raised against the particular marker protein of interest, constitutes the solid phase. Diluted patient body fluid, e.g., serum or plasma, typically about 25 μ l, standards and controls are added to separate solid supports and incubated. When the marker protein is present in the body fluid it is captured by the immobilized antibody which is specific for the protein. After incubation and washing, an anti-marker protein detector antibody, e.g., a polyclonal rabbit anti-marker protein antibody, is added to the solid support. The detector antibody binds to marker protein bound to the capture antibody to form a sandwich structure. After incubation and washing an anti-IgG antibody, e.g., a polyclonal goat anti-rabbit IgG antibody, labeled with an enzyme such as horseradish peroxidase (HRP) is added to the solid support. After incubation and washing a substrate for the enzyme is added to the solid support followed by incubation and the addition of an acid solution to stop the enzymatic reaction.

The degree of enzymatic activity of immobilized enzyme is determined by measuring the optical density of the oxidized enzymatic product on the solid support at the appropriate wavelength, e.g., 450 nm for HRP. The absorbance at the wavelength is proportional to the amount of marker protein in the fluid sample. A set

of marker protein standards is used to prepare a standard curve of absorbance vs. marker protein concentration. This method is preferred since test results can be provided in 45 to 50 minutes and the method is both sensitive over the concentration range of interest for each marker and is highly specific.

5 The assay methods used to measure the marker proteins should exhibit sufficient sensitivity to be able to measure each protein over a concentration range from normal values found in healthy persons to elevated levels, i.e., 2SD above normal and beyond. Of course, a normal value range of the marker proteins can be found by analyzing the body fluid of healthy persons. For the S100b isoform where
10 +2SD = 0.02 ng/mL the upper limit of the assay range is preferably about 5.0 ng/mL. For NSE where +2SD = 9.9 ng/mL the upper limit of the range is preferably about 60 ng/mL. For MBP, which has an elevated level cutoff value of 0.02 ng/mL, the upper limit of the assay range is preferably about 5.0 ng/mL and for Tm, which has an elevated level cutoff value of about 73 ng/mL, the assay range upper limit is
15 preferably about 500 ng/mL.

The assays can be carried out in various assay device formats including those described in United States Patents 4,906,439; 5,051,237 and 5,147,609 to PB Diagnostic Systems, Inc.

20 The assay devices used according to the invention can be arranged to provide a semiquantitative or a quantitative result. By the term "semiquantitative" is meant the ability to discriminate between a level which is above the elevated marker protein value, and a level which is not above that threshold.

25 The assays may be carried out in various formats including, as discussed previously, a microtiter plate format which is preferred for carrying out the assays in a batch mode. The assays may also be carried out in automated immunoassay analyzers which are well known in the art and which can carry out assays on a number of different samples. These automated analyzers include continuous/random access types. Examples of such systems are described in United States Patents 5,207,987 and 5,518,688 to PB Diagnostic Systems, Inc. Various automated analyzers that are
30 commercially available include the OPUS® and OPUS MAGNUM® analyzers.

Another assay format which can be used according to the invention is a rapid manual test which can be administered at the point-of-care at any location. Typically, such point-of-care assay devices will provide a result which is above or below a threshold value, i.e., a semiquantitative result as described previously.

5 It should be recognized also that the assay devices used according to the invention can be provided to carry out one single assay for a particular marker protein or to carry out a plurality of assays, from a single volume of body fluid, for a corresponding number of different marker proteins. A preferred assay device of the latter type is one which can provide a semiquantitative result for the four primary
10 marker proteins measured according to the invention, i.e., S100b, NSE, MBP and a brain endothelial marker protein, e.g., Tm. These device typically are adapted to provide a distinct visually detectable colored band at the location where the capture antibody for the particular marker protein is located when the concentration of the marker protein is above the threshold level. For a detailed discussion of assay types
15 which can be utilized according to the invention as well as various assay formats and automated analyzer apparatus see U.S. Patent 5,747,274 to Jackowski.

The invention will now be described further in detail with respect to specific preferred embodiments, it being understood that these are intended to be illustrative only and the invention is not limited to the materials, procedures, etc. recited therein.

20

EXAMPLE

A prospective observational pilot study was carried out at two tertiary care hospitals. The study evaluated thirty three patients admitted with a clinical and computed tomographic (CT) diagnosis of acute ischemic stroke. The mean age of the patients presenting with stroke was approximately 66 years (66.4 ± 16.4) with an age
25 range of from 27 to 90 years. The mean delay between the onset of symptoms and presentation to the hospital was 22 hours with a range of from 1 to 72 hours. Admission National Institutes of Health Stroke Scale and Discharge modified Rankin scale scores were recorded. Blood samples were obtained on days 1 (presentation), 3, 5 and 7 at one hospital and days 1, 2 and 3 at the second hospital. All blood samples

were centrifuged and aliquots of serum were frozen and stored at -80°C until analysis for S100, NSE, MBP and Tm.

Control subjects included one hundred three healthy blood donors (age range from 18 to 78 years; mean age 54.6 ± 15.2 years) whose blood samples were used to determine reference values for concentrations of S100 and NSE and twenty four healthy blood donors who provided samples for reference measurements of MBP and Tm concentrations.

All reference values are reported as mean $+2\text{SD}$ unless otherwise stated. The reference value for S100 in serum was 0.0067 ng/mL with a 98th percentile of 0.020 ng/mL . An elevated S100 value was taken as any concentration greater than the 98th percentile (0.02 ng/mL) of normal (normal $+2\text{SD} = 0.02 \text{ ng/mL}$).

The reference value for NSE in serum was $5.03 \pm 2.40 \text{ ng/mL}$. An elevated NSE value was any concentration greater than 2SD above normal, 9.85 ng/mL .

The reference value for MBP in serum was $0.0162 \pm 0.0019 \text{ ng/mL}$. An elevated MBP value was any concentration greater than 2SD above normal, 0.02 ng/mL .

The reference value for Tm in serum was $50.52 \pm 13.62 \text{ ng/mL}$. An elevated Tm value was any concentration greater than $+2\text{SD}$ above normal, 76.14 ng/mL .

The levels of S100 and NSE were analyzed using Exact S100 and Exact NSE Elisa Assay Kits, respectively, available from Skye PharmaTech Inc., Mississauga, Canada. The levels of Tm were analyzed with an ELISA assay available from Diagnostica Stago, 9 rue des Freres Chausson, 92600 Asnieres Sur Seine, France. The level of MBP concentration was analyzed with an ELISA immunoassay from Diagnostic Systems Laboratories, Webster, Texas, United States.

In the tables showing the data obtained "D1" indicates the first day with the first blood sample being taken at the time of presentation. Subsequent days of sample collection are indicated by D2, D3, etc. For the values of the concentrations of the markers, $+2\text{SD}$ are above the normal range. "ND" signifies that no data was obtained.

TABLE II
NSE, S100, MBP ND Tm CONCENTRATIONS IN
CLINICAL SERUM SAMPLES

CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SM-1 D1	42	Female	8.342	0.028	0.000	43.535
SM-1 D3			13.300	1.098	ND	61.946
SM-1 D5			9.622	0.060	0.238	65.859
SM-1 D7			10.710	0.066	1.725	62.177
DIAGNOSIS		Left internal carotid. CEREBRAL INFARCT (arteroembolic). 5h from onset of symptoms.				
OUTCOME		GOOD. Mild aphasia.				
SM-2 D1	55	Female	9.420	0.053	0.032	ND
SM-2 D3			5.430	0.015	0.105	ND
SM-2 D5			7.360	0.011	0.341	ND
SM-2 D7			9.906	0.008	0.124	ND
DIAGNOSIS		CEREBRAL INFARCT. Posterior circulation infarction (unknown mechanism). 20 h from onset of symptoms.				
OUTCOME		MODERATE. Dysarthria and hemiparesis.				
SM-3 D1	78	Male	12.670	0.112	0.000	92.324
SM-3 D3			14.980	0.719	1.420	101.990
SM-3 D5			28.570	1.301	4.845	119.251
DIAGNOSIS		CEREBRAL INFARCT. Total anterior circulation infarction (cardioembolic).				
OUTCOME		DEATH				
SM-4 D1	58	Male	8.520	0.008	0.000	73.913
SM-4 D3			4.406	0.028	0.147	78.286
SM-4 D5			4.888	0.024	0.265	85.881
DIAGNOSIS		CEREBRAL INFARCT. Lacunar circulation infarction (lacune).				
OUTCOME		GOOD. Mild ataxic hemiparesis.				

TABLE II						
NSE, S100, MBP ND Tm CONCENTRATIONS IN CLINICAL SERUM SAMPLES						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SM-5 D2	27	Male	9.139	0.099	2.301	59.415
SM-5 D3			5.492	0.000	0.090	53.892
SM-5 D5			11.730	0.079	7.682	68.850
SM-5 D7			11.540	0.018	10.382	68.620
DIAGNOSIS		CEREBRAL INFARCT (fibromuscular dysplasia). 48h from onset of symptoms.				
OUTCOME		MODERATE. Aphasia and hemiparesis.				
SM-6 D1	63	Male	7.029	0.000	0.000	56.883
SM-6 D3			6.455	0.020	0.000	75.985
DIAGNOSIS		CEREBRAL INFARCT (unknown mechanism). 22 h from onset of symptoms.				
OUTCOME		MODERATE				
SM-7 D1	64	Female	8.566	0.021	0.013	105.212
SM-7 D3			5.061	0.024	0.000	129.146
SM-7 D5			6.783	0.021	0.017	129.607
SM-7 D8			7.377	0.015	0.000	162.746
DIAGNOSIS		CEREBRAL INFARCT. Lacunar circulation infarction (lacune).				
OUTCOME		MODERATE. Hemiparetic.				
SM-8 D1	45	Male	15.740	0.053	0.009	37.092
SM-8 D3			21.010	0.112	0.082	35.711
DM-8 D5			15.060	0.095	0.112	38.703
DIAGNOSIS		CEREBRAL INFARCT (Right vertebral dissection).				
OUTCOME		GOOD. Minimal deficit.				

TABLE II NSE, S100, MBP ND Tm CONCENTRATIONS IN CLINICAL SERUM SAMPLES						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SM-9 D1	35	Male	11.530	0.015	0.101	ND
SM-9 D5			8.033	0.021	0.040	ND
SM-9 D7			7.336	0.002	0.000	ND
DIAGNOSIS		CEREBRAL INFARCT (unknown mechanism).				
OUTCOME		GOOD. Minimal deficit.				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-01 D1	83	MALE	6.803	0.091	0.000	185.760
SJ-01 D2			8.566	0.235	0.000	166.659
SJ-01 D3			8.689	1.143	0.000	209.234
DIAGNOSIS		CEREBRAL INFARCT (recurrent). ↑BP, renal insufficiency, MI				
OUTCOME		Severe impairment developed on second day.				
SJ-02 D1	61	MALE	14.040	0.054	0.433	476.193
SJ-02 D2			13.430	0.110	1.199	403.010
SJ-02 D3			12.890	0.247	2.625	501.739
DIAGNOSIS		CEREBRAL INFARCT (parietal infarction), renal failure, MI, CA. 48 h from onset of symptoms				
OUTCOME		First CT negative. Second CT positive (Day 3). DEATH (day 5)				
SJ-03 D1	83	MALE	10.700	0.000	0.000	75.064
SJ-03 D2			8.926	0.000	0.000	81.968
SJ-03 D3			9.000	0.000	0.000	89.793
DIAGNOSIS		CEREBRAL INFARCT (lacune). ↑BP, DM				
OUTCOME		CT positive (Day 2)				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-04 D1	70	FEMALE	10.270	0.000	0.000	134.209
DIAGNOSIS		TIA. ↑BP, DM				
OUTCOME						
SJ-05 D1	72	MALE	6.639	0.000	0.326	185.760
SJ-05 D2			10.870	0.000	0.219	136.281
SJ-05 D3			8.197	0.000	0.387	132.598
DIAGNOSIS		CEREBRAL INFARCT (lacune), renal impairment				
OUTCOME		First CT negative				
SJ-06 D1	81	FEMALE	10.440	0.001	0.086	ND
DIAGNOSIS		CEREBRAL INFARCT. Renal impairment (dialysis). 36 h from onset of symptoms				
OUTCOME						
SJ-07 D1	90	FEMALE	12.540	0.001	0.162	ND
DIAGNOSIS		CEREBRAL INFARCT. 36 h from onset of symptoms				
OUTCOME						
SJ-08 D1	81	MALE	12.450	0.749	0.017	82.198
DIAGNOSIS		HAEMORRHAGIC. 1 h from onset of symptoms				
OUTCOME		CT positive. DEATH 2 h later.				
SJ-09 D1	46	MALE	4.891	0.000	0.000	88.182
SJ-09 D2			3.913	0.000	0.000	87.722
SJ-09 D3			1.848	0.000	0.000	105.903
DIAGNOSIS		STROKE (clinically). PA within 3 h of onset of symptoms				
OUTCOME		CT negative				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-10 D1	69	FEMALE	8.303	0.000	0.000	79.437
SJ-10 D2			6.000	0.000	0.000	74.144
SJ-10 D3			3.939	0.000	0.000	68.850
DIAGNOSIS		~12 h from onset of symptoms - numbness in arms - R side facial droop; difficulty swallowing - no past Hx CVA - patient diabetic; has Hx high BP				
OUTCOME		Initial CT negative. All symptoms resolved; except patient still unable to swallow.				
SJ-11 D1	39	MALE	10.770	0.058	0.063	65.398
SJ-11 D2			12.050	0.047	0.128	69.311
SJ-11 D3			17.330	0.068	0.189	76.675
DIAGNOSIS		CEREBRAL INFARCT. ~24 h from onset of symptoms - found unconscious with R-sided neglect				
OUTCOME		CT positive (Day 1) - 3 lesions present ~2 cm - basal ganglia L side Patient still has severe weakness R side with speech impairment				
SJ-12 D1	51	FEMALE	11.700	0.000	0.067	286.100
SJ-12 D2			8.788	0.000	0.055	270.911
SJ-12 D3			11.800	0.002	0.124	226.264
DIAGNOSIS		CEREBRAL INFARCT (lacune). ~ 12 h from onset of symptoms - weakness L side, esp. L arm - facial droop and pronounced slurring of speech - Bell's Palsy L side - renal dialysis patient				
OUTCOME		CT positive (Day 1) - developed thrombocytopenia Day 2				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-13 D1	78	FEMALE	10.090	0.000	0.000	46.297
SJ-13 D2 (Haemolytic)			40.040	0.768	0.433	41.924
SJ-13 D3			4.667	0.103	0.000	36.861
DIAGNOSIS		CEREBRAL INFARCT (Left MCA CVA) + CAD, + Diabetic, Hx HTN, + family Hx CVA. ~ 19 h from onset of symptoms				
OUTCOME		Initial CT negative. Initial symptoms worsened over 48 h to R hemiplegia.				
SJ-14 D1	72	MALE	7.303	0.087	0.299	NC
SJ-14 D2			5.697	0.007	0.055	NC
DIAGNOSIS		CEREBRAL INFARCT (Left CVA). ~ 9 h from onset of symptoms - prior CVA 1989 - Hx strial fib., anticoagulated - MI 1997				
OUTCOME		Symptoms improving				
SJ-15 D1	79	MALE	5.667	0.000	0.013	ND
DIAGNOSIS		CEREBRAL INFARCT (Left CVA) - symptoms progressive over 2 wk period; worsened over 3 day period just prior to presentation at hospital.				
OUTCOME		CT negative Day 1 - condition worsening at discharge (discharged at family's request for palliative care at home)				
SJ-16 D1	90	FEMALE	20.940	0.811	5.142	52.281
SJ-16 D2			12.220	0.498	5.459	55.733
SJ-16 D3			9.424	0.253	3.377	55.503
DIAGNOSIS		Large intracerebral bleed with smaller subdural hematoma and intraventricular hemorrhage - Onset of symptoms unknown (6 to 29 h prior) - previously well; no Hx other than colon Ca 20 yr prior; on no meds at home; found collapsed				
OUTCOME		Patient continues to worsen				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-17 D1	77	MALE	10.660	0.042	0.002	ND
SJ-17 D2			8.758	0.095	0.006	ND
SJ-17 D3			12.510	0.261	0.417	ND
DIAGNOSIS		CEREBRAL INFARCT (Right CVA) - old left cerebellar infarct - sudden onset; slurred speech and L-sided weakness ~ 15 h from onset of symptoms				
OUTCOME		CT showed old CVA and new right MCA infarct				
SJ-18 D1	79	MALE	21.560	0.008	0.000	61.946
SJ-18 D2			14.390	0.218	0.814	48.598
SJ-18 D3			11.050	0.102	0.698	55.963
DIAGNOSIS		Initial CT showed bleed or cerebral edema. ~ 2 h from onset of symptoms				
OUTCOME		Aphasia and R-sided weakness				
SJ-19 D1	82	FEMALE	9.948	0.000	ND	64.248
SJ-19 D2			9.781	0.008	ND	58.955
SJ-19 D3			11.720	0.023	ND	64.248
DIAGNOSIS		TIA ~ 24 h from onset of symptoms				
OUTCOME		Slurred speech, difficulty swallowing which persists.				
SJ-20 D1	ND	MALE	26.400	0.122	0.000	32.719
DIAGNOSIS		Haemorrhagic stroke				
OUTCOME						
SJ-21 D1	74	MALE	5.828	0.016	ND	74.374
SJ-21 D2			7.423	0.063	ND	75.985
SJ-21 D3			8.436	0.286	ND	71.382
DIAGNOSIS		CEREBRAL INFARCT (left CVA)				
OUTCOME		R-sided weakness				

TABLE III						
CODE #	AGE	GENDER	NSE (ng/mL) + 2SD=9.9	S100 (ng/mL) + 2SD=0.02	MBP (ng/mL) + 2SD=0.02	Tm (ng/mL) + 2SD=73
SJ-22 D1 (Haemolytic)	63	FEMALE	18.600	0.000	0.000	ND
SJ-22 D2			9.540	0.008	0.000	ND
DIAGNOSIS		CEREBRAL INFARCT (left CVA), initial CT negative				
OUTCOME		weakness (resolving)				
SJ-23 D1	79	MALE	14.530	2.009	5.478	ND
SJ-23 D2			23.980	>3.200	8.155	ND
SJ-23 D3			27.670	2.218	7.309	ND
DIAGNOSIS		CEREBRAL INFARCT, CT positive				
OUTCOME		CT showed multiple cerebral infarcts.				
SJ-24 D1	73	MALE	20.630	0.000	0.000	74.160
SJ-24 D2			17.880	0.000	0.000	89.750
SJ-24 D3			17.880	0.000	0.000	83.290
DIAGNOSIS		TIA - sudden decrease in ability to function, word difficulties				
OUTCOME		CT negative - Discharged with diagnosis of TIA				

The analysis of S100, NSE and MBP levels in serum samples from healthy control subjects showed no relationship of levels of these proteins to age or sex. In the case of Tm, the concentrations were higher in serum samples from healthy males than in females (54.62 ± 13.62 ng/mL, 2SD above normal = 81.86 ng/mL and 43.63 ± 11.18 ng/mL, 2SD above normal = 68.74 ng/mL, respectively).

Of the thirty three stroke patients twenty six were infarcts (79%) and of these five were lacunar (15%) and four had hemorrhagic stroke (12%). Of the hemorrhagic stroke patients three had subarachnoid hemorrhage and one had an intracerebral bleed. Three patients (9%) had transient ischemic attacks (TIA).

On presentation the levels of S100 were elevated in 44% of the patients, NSE levels were elevated in 59%, MBP levels were elevated in 40% and Tm levels were elevated in 57%.

The data indicate that by measuring the four marker proteins in accordance with the invention, where any one marker was elevated, 94% of the patients could be identified on presentation. Nineteen of the twenty one non-lacunar infarcts (90%) could be identified on presentation. The remaining two patients arrived at the hospital at 22 and 72 hours respectively after onset of symptoms.

Each of Figs. 3-10 is a graphical illustration of the data obtained from a different patient of the study. The concentration levels are expressed as multiples of a reference value and were obtained by dividing the actual measured concentration values by the defined reference value for each respective marker protein, i.e., the 2SD value.

All lacunar infarcts, hemorrhagic and TIA patients were identified on presentation with 100% accuracy. All five lacunar infarcts had elevated levels of Tm on presentation. In some patients the only elevated marker protein was Tm. Referring now to Fig. 3 it can be seen that, for patient SM7, the only elevated marker protein was Tm indicating a lacunar infarct.

The three TIA patients had elevated NSE levels and normal S100 and MBP levels that stayed within the normal range. Tm was elevated in one of the TIA patients. Referring now to Fig. 4 it can be seen that for patient SM-24, Tm was slightly elevated and NSE was elevated indicating a TIA. The patient was discharged with diagnosis of TIA. Referring now to Fig. 5 it can be seen that patient SM-3 had greatly elevated levels of MBP and S100 as well as elevated levels of NSE and Tm indicating a cerebral infarct with damage spreading into the base of the brain.

In the four hemorrhagic stroke patients, the three subarachnoid hemorrhagic patients had elevated levels of S100 and NSE and a normal Tm level. In the patient with an intracerebral hemorrhagic stroke the levels of S100 and NSE were elevated and the level of MBP was elevated about 250 times. Fig. 6 illustrates that patient SJ-16 had a 250 fold increased level of MBP upon presentation as well as elevated levels of S100 and NSE and had suffered an intracerebral hemorrhage.

Fig. 7 illustrates that patient SJ-2 had elevated MBP, Tm and S100 upon presentation and that the MBP and S100 levels continued to increase with time indicating a cerebral infarct with the stroke increasing over time. An initial CAT scan upon presentation was negative and became positive only days later.

5 Fig. 8 illustrates that patient SJ-18 presented with a TIA which evolved into a stroke. Tm was in the normal range indicating that the cerebral vasculature was not compromised and thus indicating that the patient was a good candidate for thrombolysis.

Fig. 9 illustrates that patient SM-8 presented with a cerebral infarct and, with
10 Tm in the normal range, was a good candidate for thrombolysis since the endothelial vasculature was not compromised.

Fig. 10 illustrates that patient SJ-1 had a cerebral infarct and because of the elevated Tm level was at risk of hemorrhage if given thrombolytics because of the endothelial vasculature being compromised.

15 For the second serum sample obtained the levels of S100 were elevated in 73% of the stroke patients, the NSE levels in 54%, MBP levels in 64% and Tm levels in 55%. These data indicated that by measuring the four marker proteins in accordance with the invention, where any one marker was elevated 96% of the patients could be identified from the second serum sample obtained.

20 The data indicate that the levels of the protein markers in subsequent serum samples either increased or decreased depending upon whether the stroke was evolving in severity or subsiding.

Eighteen (54%) of the thirty three stroke patients had a CAT scan performed on presentation. All four hemorrhagic stroke patients were CAT positive at
25 presentation. Nine (50%) of the eighteen patients had a normal CAT at presentation which became positive days later. Eight of these nine patients who had a normal CAT on presentation had elevated levels of one or more of the four protein markers on presentation. All of the nine CAT positive patients on presentation also had elevated levels of one or more protein markers on presentation.

Peak S100, NSE and MBP levels were significantly correlated (Pearson's) with admission NIHSS scores ($p < 0.05$) and discharge modified Rankin scores ($p < 0.05$).

5 The data show that levels of S100, NSE, MBP and Tm can be easily and reliably measured in acute ischemic stroke patients and that by measuring these four marker proteins in accordance with the invention, when any one marker protein is elevated a 94% sensitivity for acute ischemic stroke can be achieved upon presentation. Further, in the hyperacute period of the evolving stroke, elevated levels of one or more of these four marker proteins appear to precede irreversible tissue
10 damage and brain edema prior to detection of such damage by CAT.

Although the invention has been described with respect to various preferred embodiments it is not intended to be limited thereto but rather those skilled in the art will recognize that variations and modifications may be made therein which are within the spirit of the invention and the scope of the appended claims.

15

CLAIMS:

1. A method for diagnosing and distinguishing stroke comprising
 - a. analyzing the body fluid of a patient to detect the presence and concentration of four markers of stroke wherein
 - i. A first marker is myelin basic protein,
 - 5 ii. a second marker is the beta isoform of S100 protein,
 - iii. a third marker is neuronal specific enolase,
 - iv. a fourth marker is a brain endothelial cell membrane protein
 - and
 - b. from the information obtained from said analyses verifying
10 whether an ischemic or hemorrhagic cerebral event has occurred and differentiating a particular type of cerebral event.
2. A method as defined in claim 1 wherein said body fluid is selected from the group consisting of blood, blood products and cerebrospinal fluid.
3. A method as defined in claim 1 wherein each of said analyses is carried out on the same sample of body fluid.
4. A method as defined in claim 1 wherein at least one of said analyses is carried out on a first sample of body fluid and at least another of said analyses is carried out on a second sample of body fluid.
5. A method as defined in claim 4 wherein said first and said second samples of body fluid are taken at different time periods.
6. A method as defined in claim 1 wherein said brain endothelial cell membrane protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7, Gamma Glutamyl Transpeptidase, P-glycoprotein and combinations thereof.

7. A method as defined in claim 1 wherein at least one of said analyses comprises contacting said body fluid with an antibody which is specific for said marker.

8. A method as defined in claim 7 wherein at least one of said analyses is carried out with an enzyme-labeled immunoassay method.

9. A method as defined in claim 1 and further including the step of analyzing said body fluid for a fifth marker protein, wherein said fifth marker protein has the same specific cell type as one of said first, second or third markers and has a higher molecular weight than said first, second or third marker which has the same
5 specific cell type.

10. A method as defined in claim 9 wherein at least one of said analyses comprises contacting said body fluid with an antibody which is specific for said marker.

11. A method as defined in claim 10 wherein at least one of said analyses is carried out with an enzyme-labeled immunoassay method.

12. A method as defined in claim 1 and further including the step of analyzing a second sample of a body fluid from said patient for said four markers, said second sample of body fluid being taken at a time subsequent to said body fluid analyzed in step a.

13. A method as defined in claim 1 wherein said steps of verifying and differentiating include comparing the concentration level detected in said analysis for each said four markers to a predefined threshold level for each said marker.

14. A diagnostic kit for diagnosing and distinguishing stroke comprising at least four antibodies which are specific for each of four different marker proteins, said antibodies immobilized on a solid support, wherein

- 10 a. a first marker protein is myelin basic protein and a first antibody is specific therefor,
- b. a second marker protein is the beta isoform of S100 protein and a second antibody is specific therefor,
- c. a third marker protein is neuronal specific enolase and a third
15 antibody is specific therefor, and
- d. a fourth marker protein is a brain endothelial cell membrane protein and a fourth antibody is specific therefor and at least four labeled antibodies, each of said labeled antibodies binding to one of said marker proteins.

15. A diagnostic kit as defined in claim 14 wherein each of said four antibodies is immobilized on the same solid support.

16. A diagnostic kit as defined in claim 14 wherein at least one of said four antibodies is immobilized on a first solid support and at least another of said four antibodies is immobilized on a second solid support.

17. A diagnostic kit as defined in claim 14 wherein at least one of said labeled antibodies comprises an enzyme-labeled antibody.

18. A diagnostic kit as defined in claim 14 wherein said brain endothelial cell marker protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7, Gamma Glutamyl Transpeptidase, P-glycoprotein and combinations thereof.

19. A diagnostic kit as defined in claim 14 and further including a fifth antibody which is specific for a fifth marker protein, wherein said fifth marker protein has the same specific cell type as one of said first, second or third markers and has a higher molecular weight than said first, second or third marker which has the same

5 specific cell type, and a fifth labeled antibody which binds to said fifth marker protein.

20. A diagnostic kit as defined in claim 19 wherein said fifth labeled antibody comprises an enzyme-labeled antibody.

21. A method for the differential diagnosis of ischemic and hemorrhagic
5 cerebral events comprising

a. analyzing the body fluid of a patient to detect the presence and concentration level of one or more ischemic marker proteins selected from the group consisting of myelin basic protein, the beta isoform of S100 protein, neuronal specific enolase and combinations thereof,

10 b. analyzing the body fluid of said patient to detect the presence and concentration level of a brain endothelial cell membrane protein, and

c. from the information obtained from said analyses, verifying the occurrence of an ischemic or hemorrhagic cerebral event and differentiating a particular type of cerebral event.

15 22. A method as defined in claim 21 wherein said steps of verifying and differentiating include comparing the concentration levels detected in said analyses for said one or more ischemic marker proteins and for said brain endothelial cell membrane protein to a predefined threshold level for each said ischemic marker protein and for said brain endothelial cell membrane protein.

20 23. A method as defined in claim 21 wherein said body fluid is selected from the group consisting of blood, blood products and cerebrospinal fluid.

24. A method as defined in claim 21 wherein said brain endothelial cell membrane protein is selected from the group consisting of Thrombomodulin, Glucose Transporter 1 in the dimeric or tetrameric form, Neurothelin/HT7. Gamma Glutamyl
25 Transpeptidase, P-glycoprotein and combinations thereof.

25. A method as defined in claim 24 wherein said brain endothelial cell membrane protein is Thrombomodulin.

26. A method as defined in claim 21 further including
30 analyzing said body fluid to detect the presence and concentration level of a secondary marker protein having the same specific cell type as one of said myelin basic protein, beta isoform of S100 protein or neuronal specific enolase whereby the time of onset of a hemorrhagic or ischemic cerebral event can be determined.

27. A method as defined in claim 26 wherein said secondary marker protein
35 has a higher molecular weight than said myelin basic protein, beta isoform of S100 protein or neuronal which has the same specific cell type.

28. A method as defined in claim 21 wherein each of said analyses is carried on the same sample of body fluid.

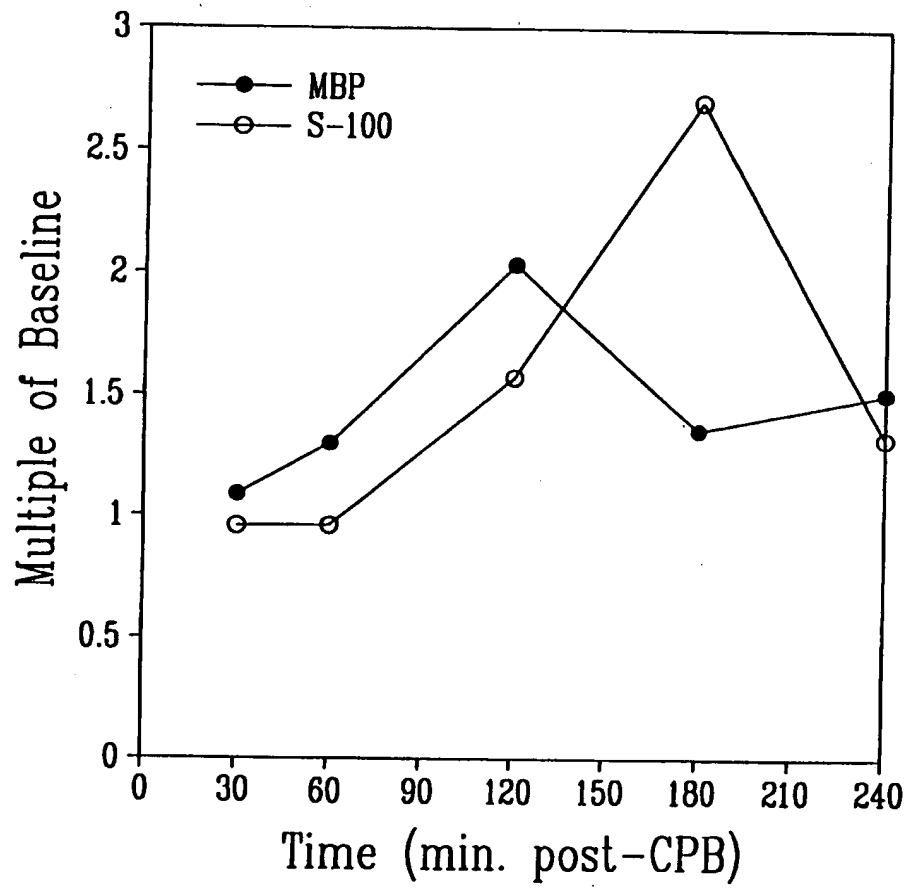
29. A method as defined in claim 21 wherein at least one of said analyses is
40 carried out on a first sample of body fluid and at least another of said analyses is carried out on a second sample of body fluid.

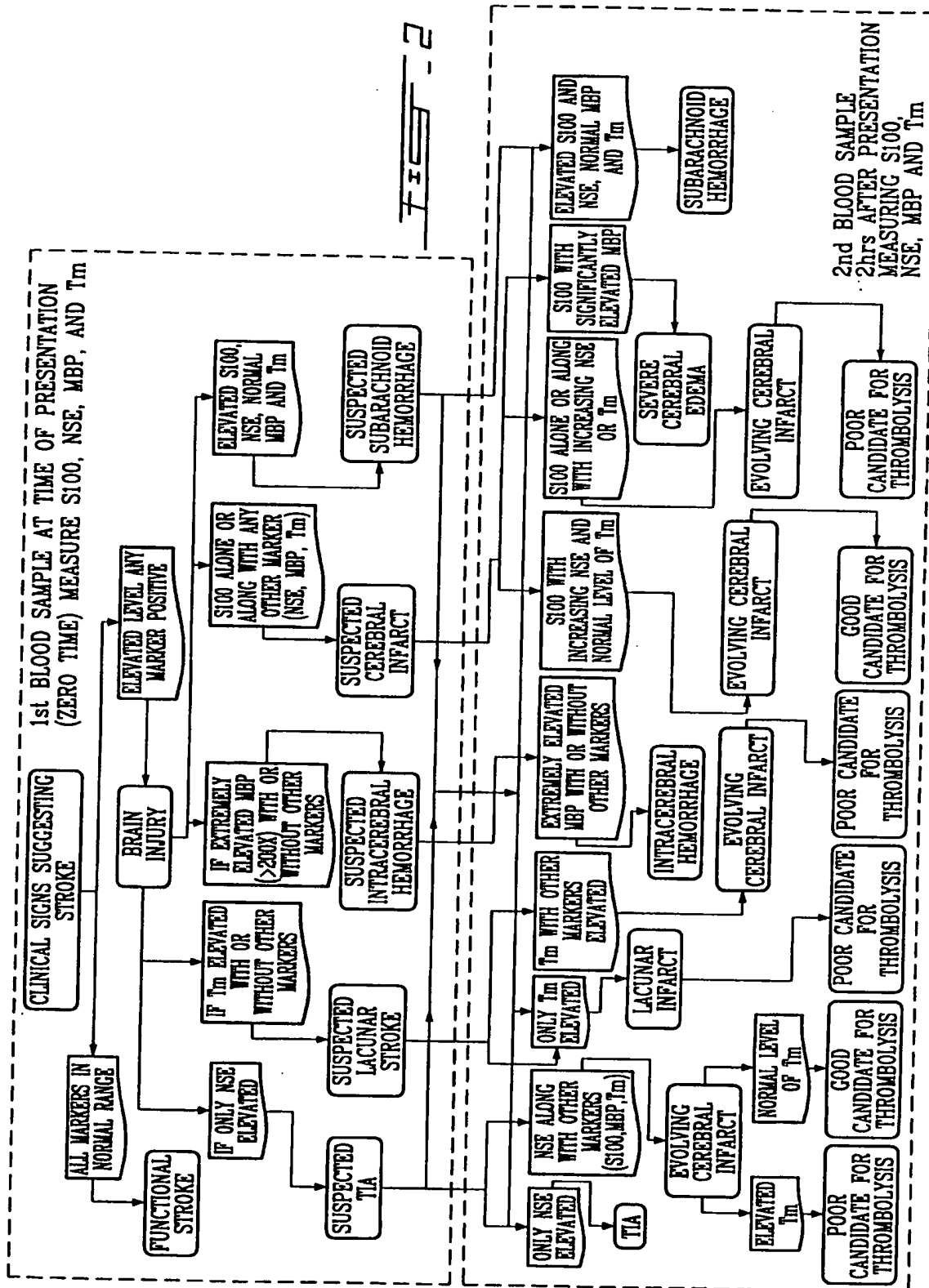
30. A method as defined in claim 29 wherein said first and said second samples of body fluid are taken at different time periods.

31. A method as defined in claim 21 wherein
45 a plurality of samples of said body fluid are obtained at predefined time intervals and analyzed and the information from said analyses compared as a function of time whereby the progression of an ischemic or hemorrhagic cerebral event can be determined.

32. A method as defined in claim 21 wherein each of said analyses comprises
50 contacting said body fluid with an antibody which is specific for said protein.

33. A method as defined in claim 32 wherein at least one of said analyses is carried out with an enzyme-labeled immunoassay method.

Figure 1



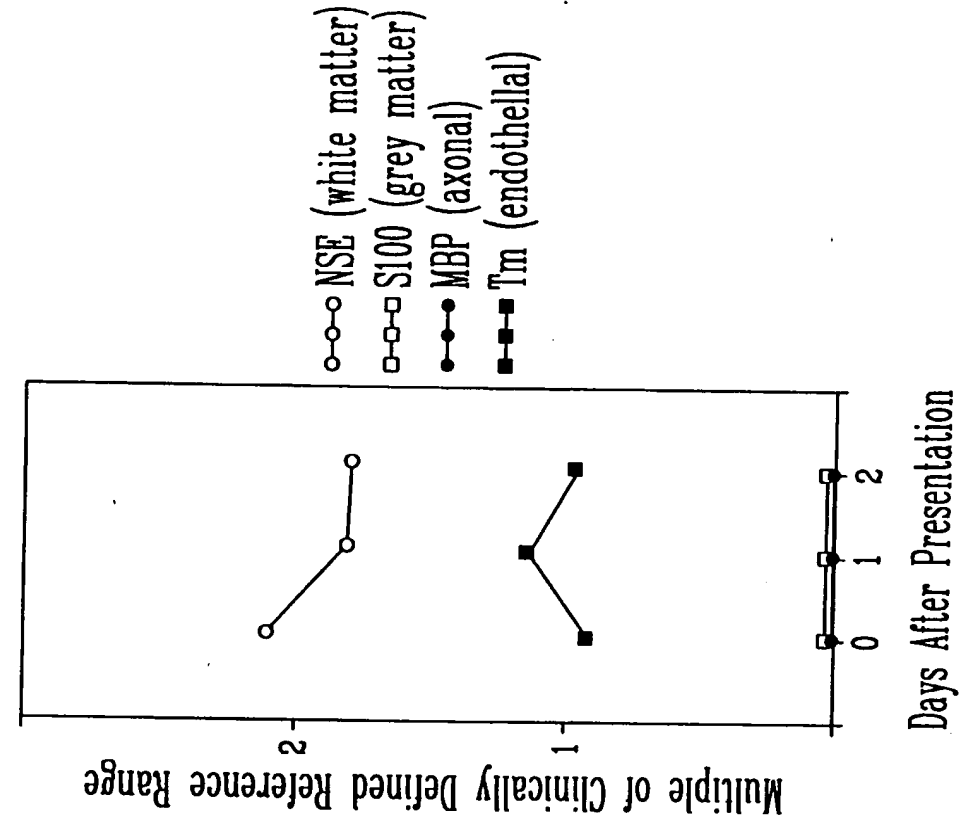


Figure 4

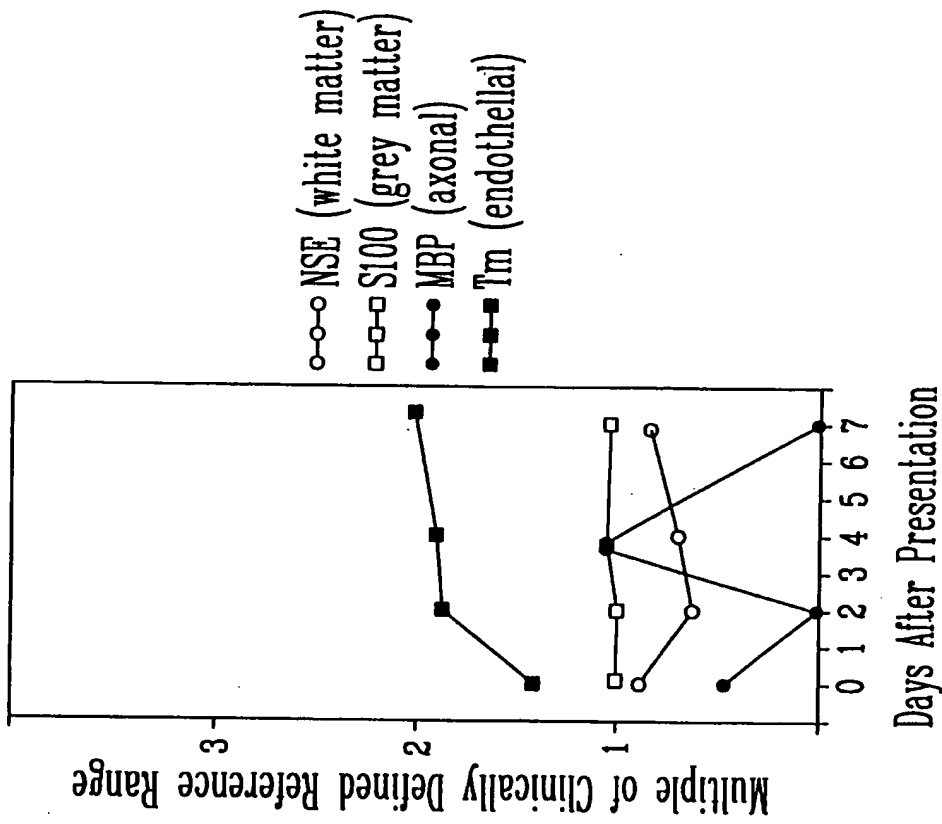
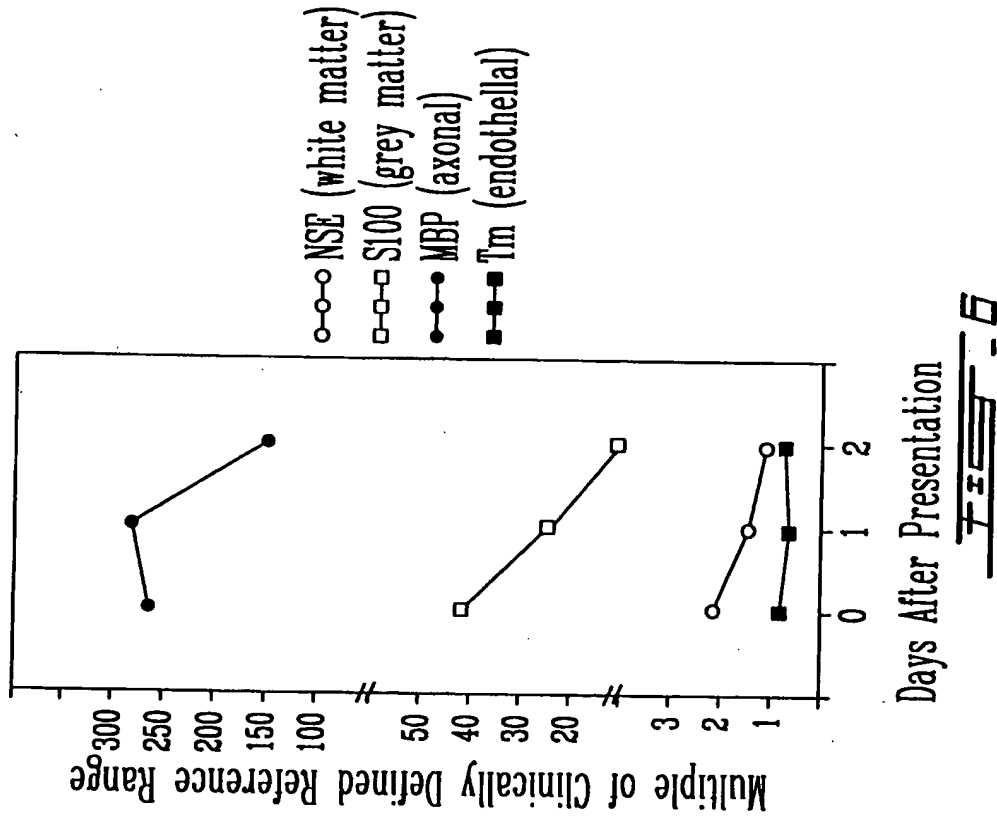
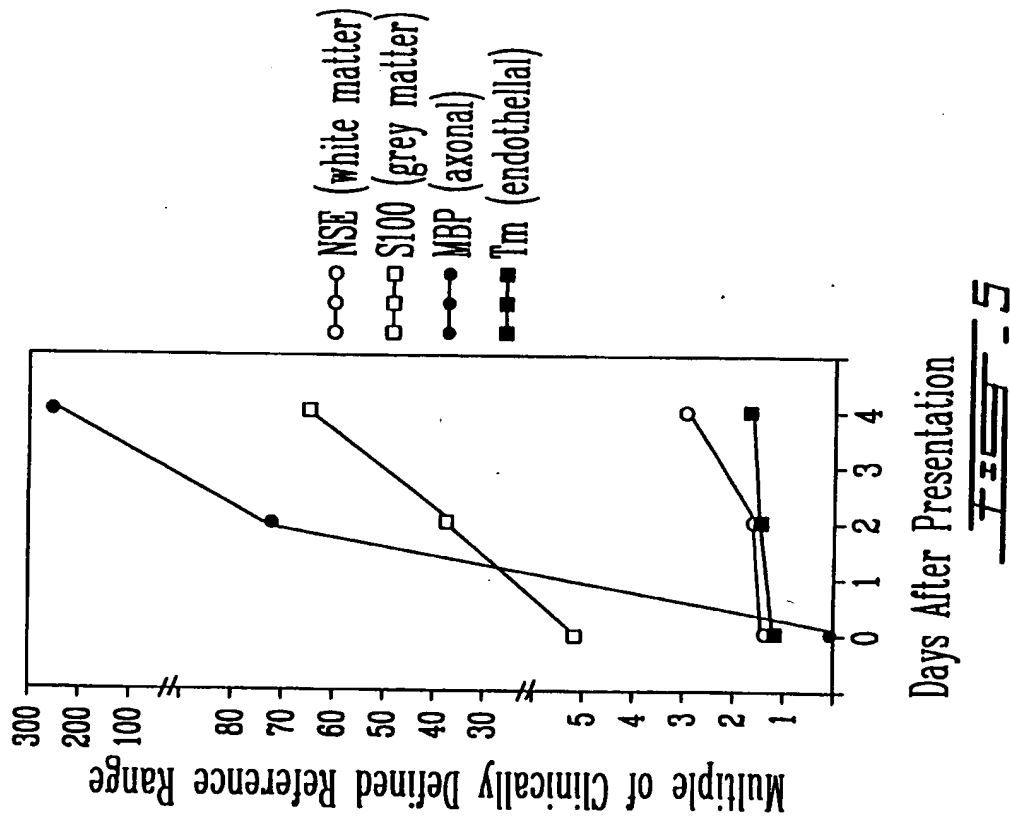
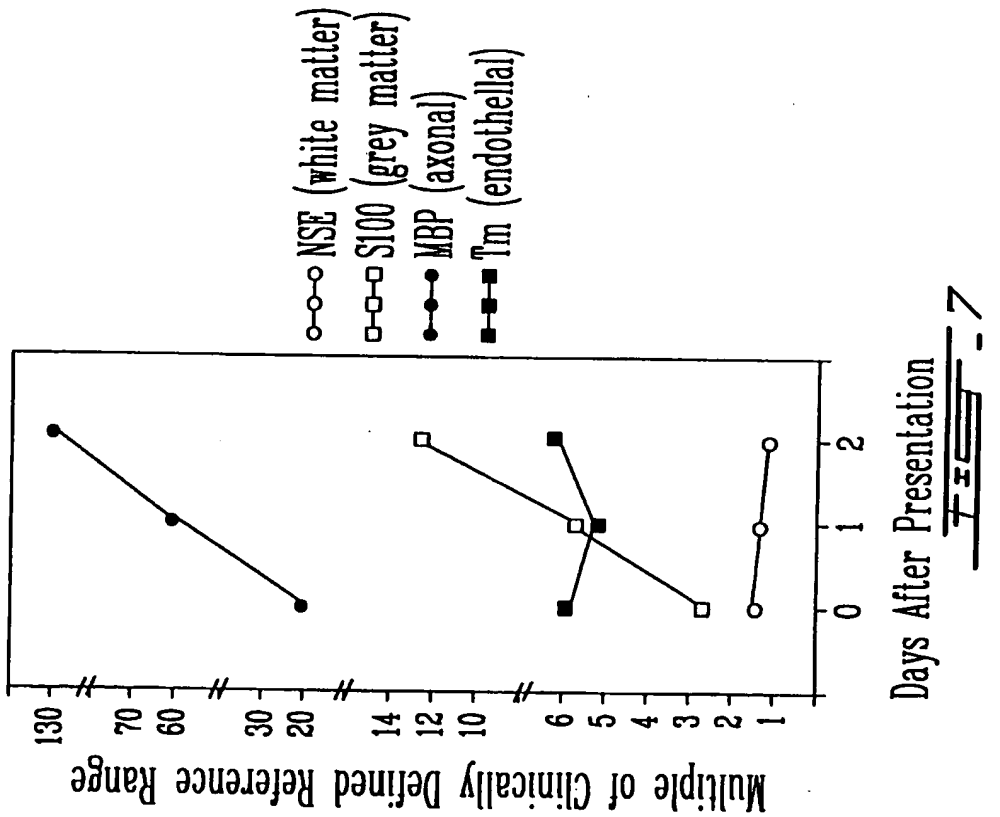
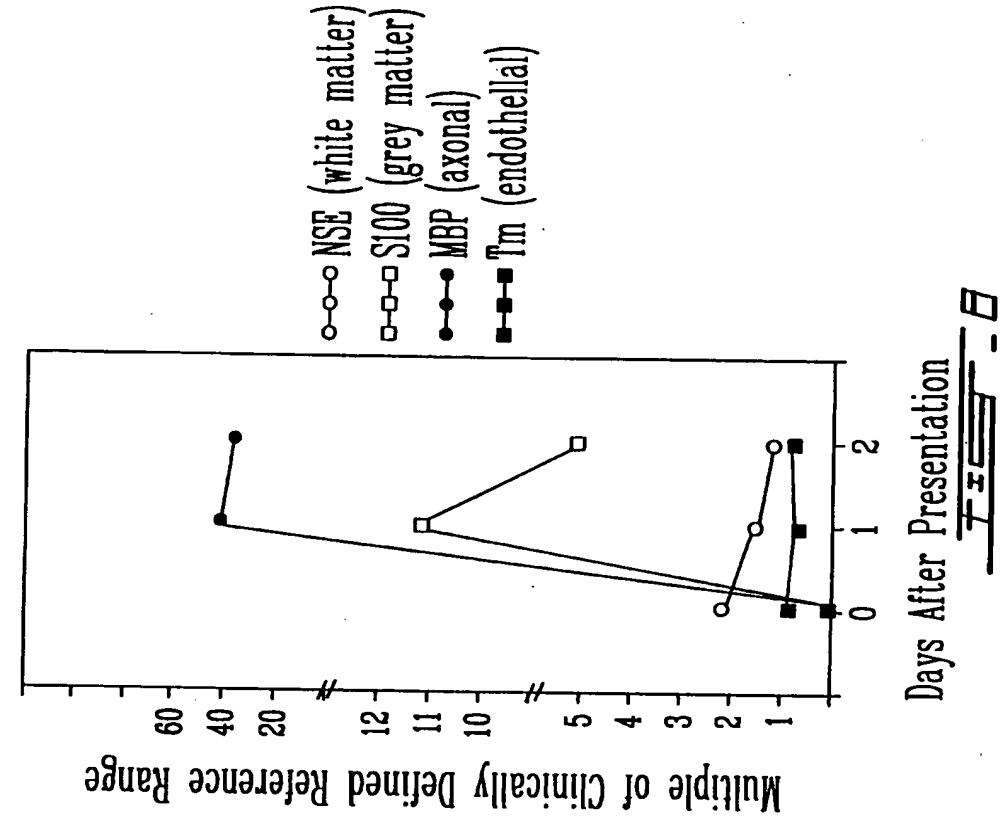
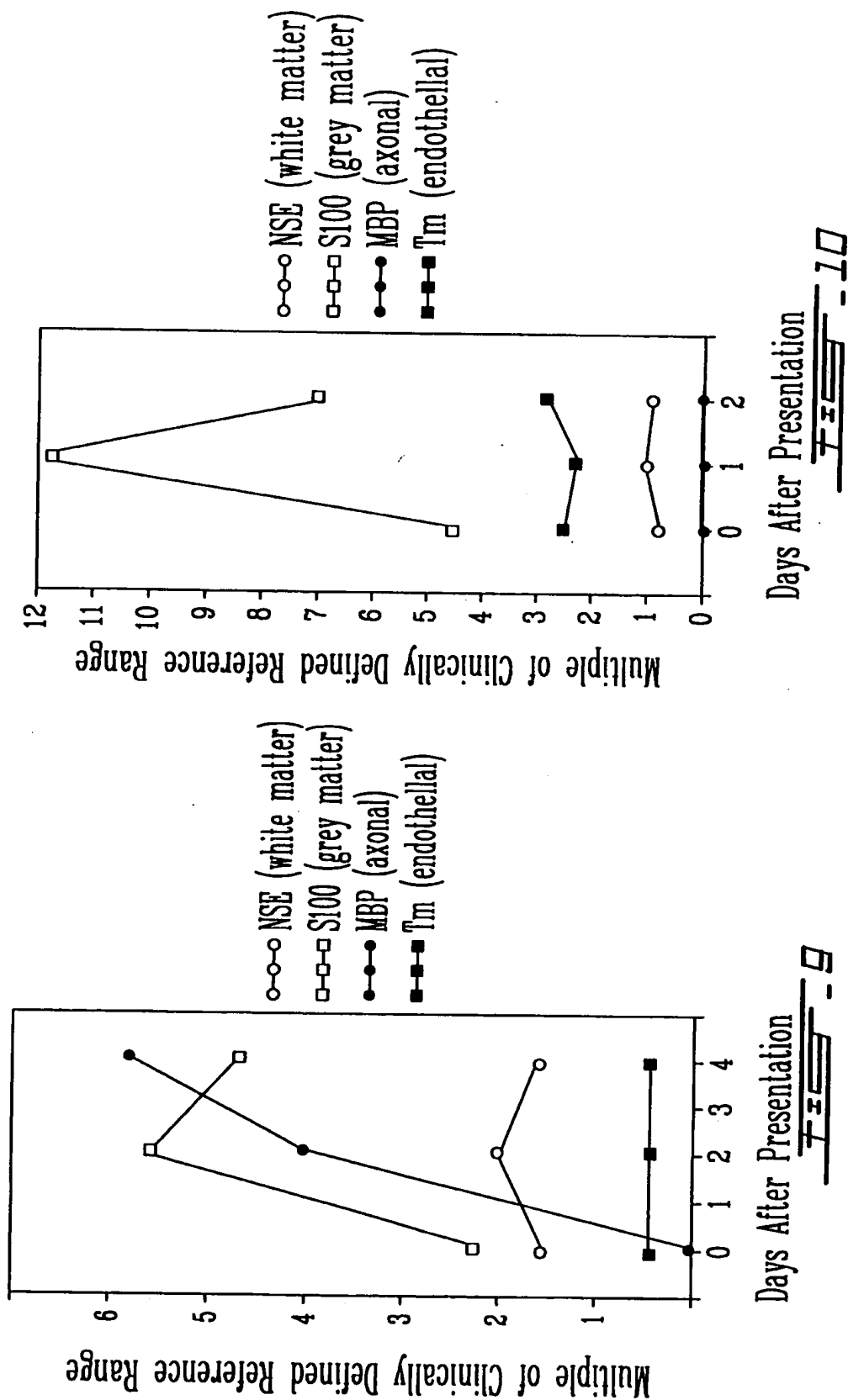


Figure 3







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Inventor: **VALKIRS, GUNARS**

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